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Characterization of solid and dissolved carbon in a spruce-fir Spodosol

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Abstract. Organic substances are an integral part of the biogeochemistry of many elements in forest ecosystems. However, our understanding of the composition, chemistry, and reactions of these materials are incomplete and sometimes inconsistent. Therefore, we examined in detail dissolved organic carbon (DOC) in forest floor leachates over a two-year period (1992–1993), soil C, and DOC adsorption by a mineral soil to determine the relationship between soil solid and solution C characteristics in a spruce-fir ecosystem. The structural composition of DOC, DOC fractions (hydrophobic and hydrophilic acids, hydrophilic neutrals), and soil samples from the organic and mineral horizons were also analyzed using ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

Total DOC in forest floor leachates ranged from 7.8 to 13.8 mmol L^{-1} with an average of 8.6 mmol L^{-1}. Concentrations were highest in September of both 1992 and 1993. Fractionation of the forest floor DOC indicated these solutions contained high organic acid contents that averaged 92% of the total DOC. Hydrophobic acids were also preferentially adsorbed by the B horizon. The ^{13}C NMR data suggested alkyl, carbohydrate, aromatic, and carboxylic C were the primary constituents for organic and mineral soils, DOC, and DOC fractions. Compositional changes of C were observed as aromatic and carbohydrate decreased, whereas alkyl, methoxy, and carbonyl moieties increased with depth. However, C composition changed little among the three organic layers based on the similarity of alkyl/carbohydrates ratios as determined from NMR area integration, suggesting that in this acid soil, decomposition proceeds rather slowly. Hydrophobic acids contained high contents of aromatic C, whereas hydrophilic acids were comprised primarily of carboxylic C. Hydrophilic neutrals were rich in carbohydrate C. Results indicated that these DOC fractions were unaltered during the isolation process. Carboxylic C groups appeared to dissolve easily and were probably the primary contributor to organic acidity in our organic dominate leachates. Results also suggested that DOC materials adsorbed on the B horizon underwent further biodegradation. Several seasonal patterns of C composition were observed in the forest floor leachates and DOC fractions collected between 1992 and 1993.

Overall, the evidence from this study suggested that (i) DOC levels were mainly controlled by biological activity, (ii) forest floor DOC was comprised primarily of organic acids, (iii) contact of soil leachates with B horizon material affected DOC quantitatively and qualitatively, (iv) phenolic, carboxylic, and carbonyl C appeared to dissolve readily in the forest Oa horizon, (v) DOC materials adsorbed on the B horizon selectively underwent further decomposition, and (vi) C composition is a function of the extent of decomposition and DOC fractions.

Introduction

The chemical composition and reactions of organic materials in forest soils have only recently been studied. The cycling of major nutrients (N, P, and S) in forest soils is dependent on organic transformation processes and mechanisms (David et al. 1995). For example, Qualls (1989) estimated that over 90 and 66%, respectively, of dissolved N and P leached from a deciduous forest soil profile were in organic forms.

Recent studies have evaluated the movement of DOC from the forest floor, DOC composition, and its reactivity with the mineral soil (David & Vance 1991; David et al. 1989; David et al. 1992; David & Zech 1990; McDowell & Likens 1988; McDowell & Wood 1984; Vance & David 1991a). Many of the important links among DOC fluxes and concentrations, forest floor, and mineral soils have been addressed. Other studies, such as Vance & David (1989), have investigated the effect of acidic deposition on the solubilization and movement of natural organic acids. Concentrations of DOC in forest floor leachates were found to vary significantly over time in some cases. For example, DOC concentrations ranged from 2.2 to 7.2 mmol L^{-1} in a hardwood forest floor (Vance & David 1991b). These leachates were comprised of organic acids (i.e., hydrophobic and hydrophilic acids totaled 92% of the DOC), with hydrophobic acids being the larger fraction at 60%. Although the DOC leached from the forest floor can be quite variable, many studies (e.g., Guggenberger & Zech 1993; McDowell & Likens 1988; McDowell & Wood 1984; Vance & David 1989) have shown that the spodic horizons adsorbed DOC, which results in low and uniform concentrations of DOC in mineral horizons. More importantly, this suggests the material that is leached from the forest floor is relatively stable and first removed by sorption, followed by slow decomposition over time.

The role of soil organic materials in nutrient cycling will not be fully understood without considering the chemical and structural changes that occur during decomposition processes operating in forest soils. Therefore, forest humus profiles have been investigated by a combination of wet-chemical degradation methods and solid ^{13}C NMR spectroscopy (deMontigny et al. 1993; Hempfling et al. 1987; Kögel et al. 1988; Kögel-Knabner et al. 1988, 1990; Zech et al. 1985, 1990). The solid state CP/MAS (cross polarization and magic angle spinning) ^{13}C NMR technique (Malcolm 1990; Schnitzer 1990; Wilson 1990) offers the advantages of direct, nondestructive structural characterization of organic materials. The composition of C in isolated organic acid fractions has been measured by solution-state ^{13}C NMR using samples collected from a northern hardwood forest (Vance & David 1991b), and recently by solid-state ^{13}C NMR using Norway spruce forest samples (Guggenberger et al. 1994).

Due to the importance of organic materials in forest nutrient cycles, a better understanding of C solubilization and leaching from the forest floor (organic horizons) is needed. The objectives of this study were to: 1) characterize the soil solid and solution C chemistry in a spruce-fir ecosystem; 2) investigate changes that occur in ^{13}C NMR patterns with soil depth; 3) evaluate patterns and changes in DOC composition using ^{13}C NMR examination of freeze-dried forest floor bulk sample and its DOC fractions over a two-year period; and 4) use this information to determine how decomposition processes alter C forms in soils and soil solutions.

Materials and methods

The study site was located at Howland, Maine (45°10′ N, 68°40′ W) within the International Paper Company's Northern Experiment Forest. A 0.1-ha plot was established between two 0.2-ha nutrient cycling plots that were used in previous studies (Fernandez et al. 1990; Lawrence & Fernandez 1991). Red spruce (*Picea rubens* Sarg.) is the dominant species in an overall cover type of spruce-fir, with white pine (*Pinus strobus* L.) and eastern hemlock (*Tsuga canadensis* (L.) Carr.) also present. Few large (>4 cm) balsam fir (*Abies balsamea* (L.) Mill.) are present at the site due to damage from the last spruce budworm (*Choristoneura fumiferana* Clem.) infestation (Lawrence and Fernandez 1991). Stand age is uneven (45–130 years) because of single-tree selective logging. The site has a flat topography with an elevation of 60 m and a continental climate. Within the study plot, soils have developed on the dense basal till and are classified as Aquic Haplorthods. Additional soils information has been reported in studies by Lawrence & Fernandez (1991) and Fernandez et al. (1993).

Five soil pits were established in the experimental plot and sampled by horizon. Subsamples of soils from each horizon were thoroughly mixed, frozen, freeze-dried, and sieved (2 mm), and the remaining material air dried and sieved (2 mm). Analyses on air-dried soil included: pH in 0.01 M $CaCl_2$ using soil-to-solution (w/v) ratios of 1:2 for mineral horizons and 1:10 for organic horizons; total C by dry combustion using a LECO CR-12 analyzer (Nelson & Sommers 1982); and total N by semi-micro Kjeldahl digestion (Bremner & Mulvaney 1982) followed by detection of ammonium with a Wescan ammonia analyzer (Scott et al. 1989). Air-dried values were normalized (dry mass basis) after drying subsamples at 105 °C and 65 °C for mineral and organic samples, respectively. Table 1 summarizes select chemical characteristics of the sample horizons.

To collect large volumes of leachate for DOC evaluation, two PVC collectors (90 cm wide, 120 cm long, and 12 cm deep) were installed beneath

Table 1. Selected chemical characteristics of soil horizons from a spruce-fir site at Howland, Maine (Aquic Haplorthod). Values are means and standard errors (in parentheses) based on four samples per horizon.

Horizon	pH 0.01 M $CaCl_2$	Total C	Total N	C/N mol mol^{-1}
		------- g kg^{-1} -------		
Oi	3.52 (0.05)	497 (17)	7.6 (0.7)	76.3 (9.0)
Oe	3.27 (0.11)	517 (12)	10.3 (0.2)	58.5 (1.3)
Oa	2.55 (0.05)	488 (5.6)	9.2 (0.2)	61.9 (2.0)
E	3.12 (0.09)	5.6 (0.6)	0.3 (0.01)	23.3 (3.8)
Bh	4.23 (0.08)	44.9 (4.2)	1.6 (0.3)	32.7 (4.2)
Bhs	4.79 (0.09)	19.3 (3.5)	0.6 (0.01)	34.6 (2.8)
BC	5.12 (0.10)	4.2 (1.1)	0.2 (0.05)	19.6 (1.7)
C	5.09 (0.07)	2.6 (0.3)	0.1 (0.02)	21.7 (1.7)

the forest floor in the study plot (see Vance & David 1991a for details on the use of PVC collectors). The collectors were installed in May of 1992 with weekly collections beginning in June of 1992. Volumes ranged from 0 to 6 L each week from each collector, and continued until late November when the soils froze. Solutions were shipped cold in insulated boxes to the University of Illinois and filtered through 1.2-μm glass fiber filters (Whatman GF/C), and stored at 3 °C. For each sample, DOC was determined by measuring CO_2 IR absorbence after UV-persulfate oxidation (Dohrmann DC-80 Carbon Analyzer, Santa Clara, CA).

A portion of the leachate DOC was fractionated following a modification of the method of Leenheer & Huffman (1979) and Leenheer (1981). Five fractions of DOC were characterized in this modified procedure: hydrophobic acids and neutrals and hydrophilic acids, bases, and neutrals. Detailed information on the procedure used and fraction characteristics in general can be found in Vance & David (1991a,b).

The dominant forms of DOC (hydrophobic and hydrophilic acids, hydrophilic neutrals) were then isolated using the scheme detailed in Vance & David (1991b) with one exception. The anion exchange resin (Duolite A-7, still used in fractionation procedure) was replaced with an XAD-4 resin as to retain hydrophilic acids following the removal of hydrophobic compounds using an XAD-8 resin. The combined XAD-8 and XAD-4 resin tandem procedure was selected based on the recent recommendation by Malcolm & MacCarthy (1992). The XAD-4 resin has been reported to removed nearly as much of the hydrophilic acids (85–90%) and eliminated the need for extensive hydrophilic acid cleanup due to the presence of inorganic anions. In this study, we used an XAD-4 resin volume of twice that of the XAD-8. Accordingly, we obtained recoveries ranging from 72–98% (average 86%) of the sorbed hydrophilic acids.

To further examine DOC movement at our study site, sorption was examined using the same Bhs horizon used for NMR analysis. Forest floor leachate from the October 1992 bulk sample was diluted (0.02, 2.37, 4.89, 7.39, and 9.42 mmol C L^{-1}) and added to freeze-dried soil (4 g dry-mass soil with 40 mL of solution). Samples were gently mixed end-over-end for 24 h on a slowly rotating mixer (6 rpm). After equilibration, samples were centrifuged at 1500 rpm for 10 min and then filtered through polycarbonate membrane filters (0.4 μm). Each solution was then analyzed for DOC, and the 0.02, 4.89, and 9.42 mmol L^{-1} samples further studied by DOC fractionation analysis on the final solutions.

The initial mass isotherm approach was used to interpret solute-soil interactions for different solutes and soil types (Nodvin et al. 1986a,b). Recently, several studies have demonstrated the advantages and usefulness of the initial mass isotherm in examining the adsorption of DOC and DOC fractions in A, E, B, and C horizons of forested soils (David & Zech 1990; Donald et al. 1993; Guggenberger & Zech 1992, 1993; Vance & David 1989, 1992).

The initial mass isotherm describes the relationship between the amount of DOC adsorbed or released (RE) to the initial amount of DOC added with respect to the mass of soil (Xi)

$$\mathrm{RE} = \mathrm{mXi} - \mathrm{b} \qquad [1]$$

The linear regressed slope (m) represents the partition coefficient and the intercept (b) describes the unretained or desorbed portion of the reactive soil pool. The reactive soil pool (RSP) is the amount of substance present in the soil that can be readily solubilized or released. The RSP is calculated as

$$\mathrm{RSP} = \mathrm{b}/(1 - \mathrm{m}) \qquad [2]$$

A distribution coefficient (Kd), which measures the affinity of the substance for the soil is calculated as

$$\mathrm{Kd} = \mathrm{m}/(1 - \mathrm{m}) * [(\text{solution volume})/(\text{soil mass})] \qquad [3]$$

Forest floor leachates were bulked on a monthly basis and 14 L used for DOC isolation. Samples from eleven months (June to October, 1992 and June to November, 1993) were isolated, with replicate samples isolated in August 1992 (two 14 L samples). After isolation, recovered and protonated acids and neutrals were freeze-dried and stored at 3 °C for NMR analysis. In addition, a sample (4 to 6 L) of the monthly bulk solution used for isolation was passed through a cation-exchange column and freeze-dried. Therefore, for each month we had large, freeze-dried samples of the bulk solution, hydrophobic acids, hydrophilic acids, and hydrophilic neutrals (some monthly samples were bulked to obtain enough C). Amounts of recovered C ranged from 704–1074 and 63–169 mg in hydrophobic and hydrophilic acid samples, respectively.

Composition of C in the isolated organic acid fractions, bulk forest floor leachates, and some soil samples from organic and mineral horizons were analyzed by semi-quantitative solid state CP/MAS ^{13}C NMR with sideband suppression (Dixon sequence) on a General Electric GN 300WB spectrometer. For freeze-dried solution samples, 3600 to 8000 scans were taken for each spectrum; for forest floor samples, 3600 to 6400 scans were obtained, and the Bhs horizon sample required 57,000 scans. Additional conditions were: spectrometer frequency, 75.5 MHz; spectral width, 32 KHz; spinning speed, 5 KHz; contact time, 1 ms; external standard of hexamethylbenzene; pulse delay, 1 s; number of points for decay curve, 1024; line broadening factor, 100 Hz.

Detailed information about the theory of solid-state ^{13}C NMR applicable to geochemistry and soil chemistry aspects can be found in Wilson (1987) and Pfeffer & Gerasimowicz (1989). Wilson (1990) and Baldock & Preston (1995) also stated some important considerations when quantitatively interpreting the solid-state ^{13}C NMR spectra. Nonetheless, the incorporation of spinning sideband suppression techniques with CP/MAS (Dixon et al. 1981a,b) has been shown to work reasonably well on soil components (Wilson 1987).

Results and discussion

DOC concentration and composition

Concentrations of DOC in bulk forest floor leachates varied significantly over the annual cycle, with an average of 8.6 mmol C L^{-1} and a range of 7.8 to 13.8 mmol C L^{-1} (Figure 1). A seasonal trend was observed with DOC levels peaking in both September 1992 and September 1993, and decreasing as soil temperatures decreased. During the summer growing season, biological activity was assumed to follow soil temperature and resulted in increases in DOC concentrations, with both temperature and DOC peaking in September. This seasonal trend corresponds with the results of Guggenberger & Zech (1993) who reported that DOC concentrations in the organic horizon was greatest following a warm temperature period and attributed this increase to enhanced mineralization (Guggenberger 1992; Matzner & Thoma 1983). A similar time series pattern of DOC concentrations collected from O/A horizon leachates was reported by Cronan & Aiken (1985).

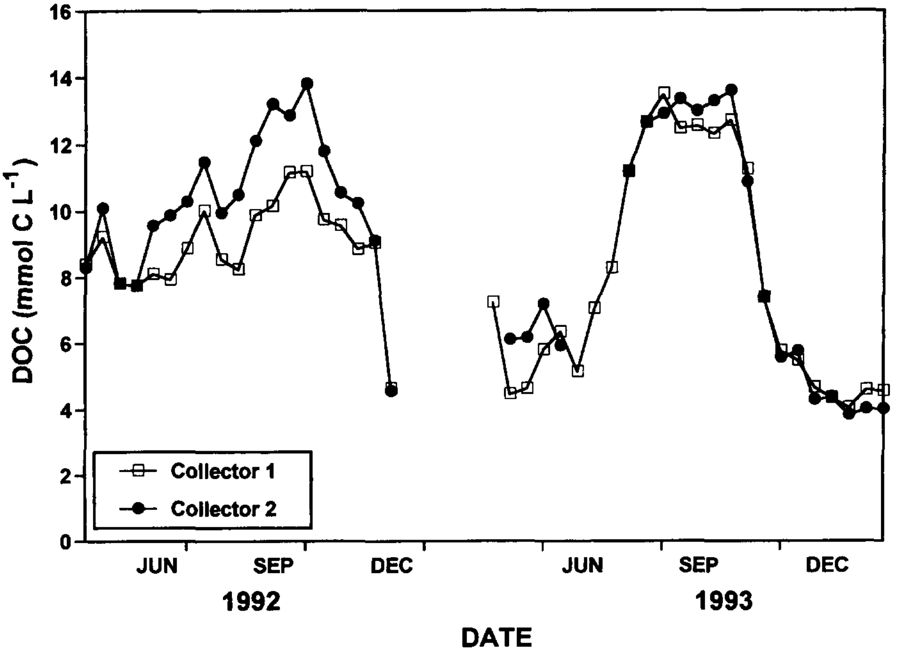


Figure 1. Dissolved organic carbon (DOC) from two forest floor collectors located at the Howland spruce-fir study site.

Bulk forest floor leachates were composed primarily of organic acids, particularly hydrophobic acids, based on DOC fractionation analysis (Figure 2). On average, the sum of organic acids accounted for 92% of the DOC, with hydrophobic acids being the larger fraction at 68%. The remaining DOC fractions (bases plus neutrals) reached a maximum of 17% of the DOC. Of these fractions, hydrophobic neutrals and hydrophilic bases were dominant. Several studies on hardwood and conifer forest floor leachates reported similar acid fractions predominance (hydrophobic acids: 46 to 60%; hydrophilic acids: 29 to 43%) (Cronan & Aiken 1985; Vance & David 1991b; Yavitt & Fahey 1985a,b). However, we measured slightly larger fractions of hydrophobic acids and smaller fractions of hydrophilic acids as compared to results reported by other investigators. This difference was assumed to be due to the higher DOC concentrations obtained in our field collectors. Concentrations of DOC in our forest floor leachates were approximately two- to five-fold more concentrated than reported in other studies; the proportion of hydrophobic acids has been suggested to increase as DOC concentration increases (David & Vance 1991). In contrast, Antweiler & Drever (1983) reported that hydrophilic acid fractions were larger (43 to 54% of total DOC)

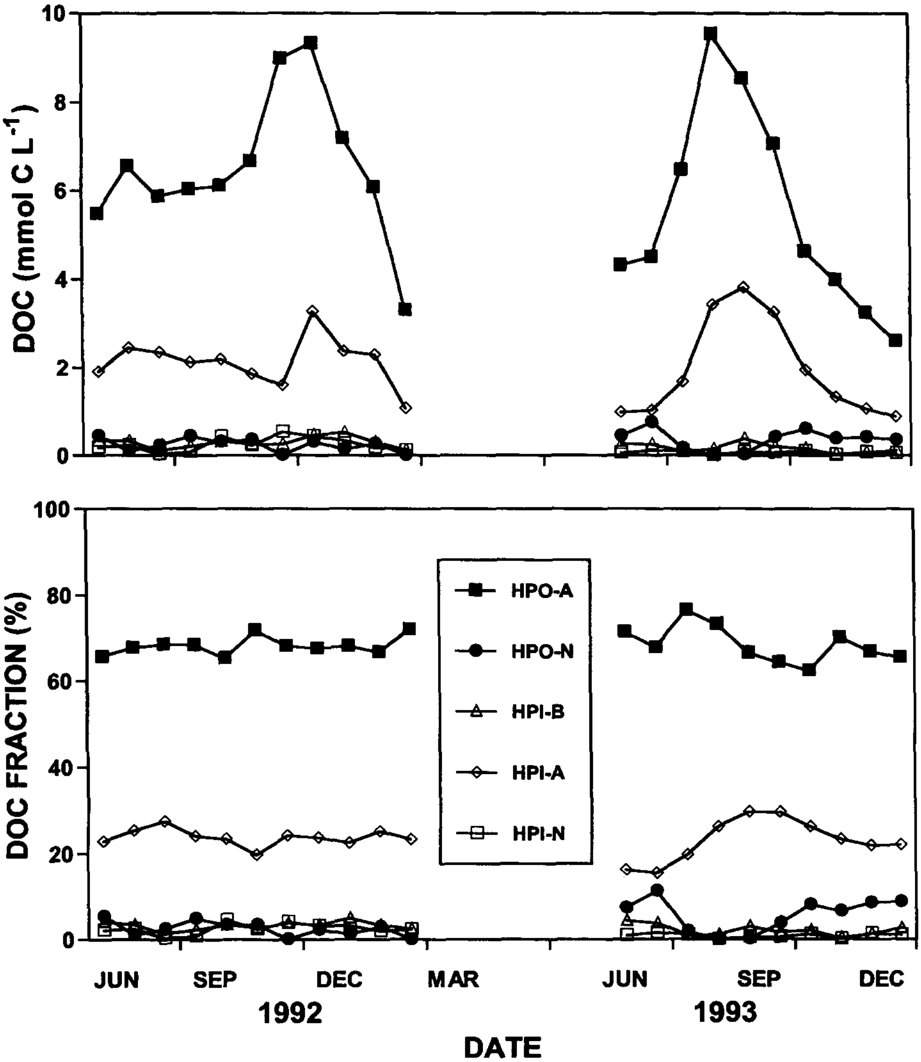


Figure 2. Percentage and absolute concentration of DOC fractions from forest floor leachates collected over two years. Legend abbreviations represent: HPO-A, hydrophobic acids; HPO-N, hydrophobic neutrals; HPI-B, hydrophilic bases; HPI-A, hydrophilic acids; HPI-N, hydrophilic neutrals.

than hydrophobic acid fractions (14 to 39% of total DOC) in grasses and hardwood leachates (50-cm deep) beneath buried volcanic ash. The ratios of hydrophobic to hydrophilic acids varied from 2.2 to 4.4 with an average ratio of about 3.0. The ratios were higher when leachate solutions were less acidic (ratios 3.6–4.4 at pH 4.1–4.2) compared to more acidic leachate solutions (ratios 2.2–3.4 at pH 3.6–3.8). These results are consistent with those of the laboratory leaching study by Cronan (1985) and field-collected soil extracts by David et al. (1989). Previous studies (Cronan & Aiken 1985; Vance & David 1991b) have reported ratios as low as 1.1 to 1.3 in the summer for hardwood forest floor leachates. However, we observed a decrease in the ratio of hydrophobic to hydrophilic acids during fall months of 1993 (Figure 2). A similar trend has been reported and suggested to be related to increasing activity of saprophytic organisms due to warmer soil temperatures (Vance & David 1991b).

DOC fractionation and isolation

A comparison of DOC analytical fractionation and preparative isolation results indicated that the proportion of major DOC fractions (hydrophobic acids, hydrophilic acids, and hydrophilic neutrals) were different (Table 2). The percent hydrophobic and hydrophilic acids were lower in the isolation scheme than the fractionation scheme. In particular, the XAD-4 resin removed only about 60% of the hydrophilic acids, compared to the cation exchange resin (Table 2). Any unretained hydrophilic acids in the isolation procedure were then classified as hydrophilic neutrals. Also, hydrophilic bases were not recovered in the isolation procedure and thus were accounted for in the neutral fraction. These differences were probably due to the secondary cleanup procedure used in the isolation method and the resins involved. As stated, the XAD-4 resin used in the isolation method is known to recover smaller amounts of hydrophilic acids as compared to the anion exchange resin used in the fractionation procedure (Malcolm & MacCarthy 1992). Overall, however, results of both methods showed that acid fractions are dominant in the forest floor solutions. In addition, the isolation scheme provided large amounts of these acids, which were examined both chemically and by ^{13}C NMR.

DOC duplicate isolation

The DOC fraction results of duplicate bulk leachates (from August 1992) that were isolated indicated that they were quite similar (Table 2). Because the isolation analysis is so time consuming and requires such large volumes of solutions, duplicate analysis has not been reported to our knowledge in previous studies on DOC characterization. Our results suggest that the isolation procedure used here is highly reproducible in terms of the measured DOC fractions and, therefore, the isolates obtained.

Table 2. Proportion (% of total) of DOC fractions as determined by fractionation and isolation schemes.

SAMPLE	FRACTIONATION					ISOLATION			
	Hydrophobic		Hydrophilic			Hydrophobic		Hydrophilic	
	Acids	Neutrals	Bases	Acids	Neutrals	Acids	Neutrals	Acids	Neutrals
July 92	70.1	2.1	3.4	21.5	2.9	59.1	8.8	6.3	25.8
Aug 92	69.1	5.5	3.1	20.2	2.1	60.7	0.0	12.8	26.5
Aug 92[1]	nd	nd	nd	nd	nd	60.7	0.0	13.2	26.1
Sept 92	71.1	1.5	3.5	21.6	2.3	59.6	5.7	10.4	24.3
Oct 92	67.9	3.5	3.4	22.6	2.6	57.8	5.7	12.5	24.0
June 93	71.4	7.3	4.3	16.2	0.8	75.8	1.4	13.0	9.8
June 93	67.9	11.3	3.7	15.5	1.6	69.3	5.0	12.2	13.5
June 93	66.3	10.9	3.0	18.3	1.5	69.2	4.8	13.2	12.8
July 93	76.5	1.8	0.9	19.7	1.1	70.6	3.6	10.7	15.1
Aug 93	66.7	0.1	2.9	29.8	0.5	54.6	2.9	16.6	25.9
Sept 93	64.4	3.7	1.6	29.6	0.5	54.5	5.3	15.1	25.1
Sept 93	62.5	8.0	2.0	26.3	1.1	52.8	8.5	17.2	21.5
Oct 93	70.2	6.5	0.3	23.0	0.0	62.5	6.7	17.0	13.8
Oct 93	67.0	8.5	1.3	24.5	1.3	63.2	6.7	15.4	14.7
Nov 93	65.6	8.7	2.6	22.1	1.0	66.3	1.7	16.6	15.4
Avg.	68.3	5.7	2.6	22.2	1.4	62.9	4.0	13.5	19.6
Std. Error	0.9	1.0	0.3	1.1	0.2	1.7	0.9	0.8	1.6

[1] Duplicate sample, nd = not determined.

Adsorption of DOC and DOC fractions

The DOC solutions added to the Bhs horizon were readily adsorbed (Figure 3a). In addition, sorption of DOC fractions demonstrated that the hydrophobic acids were selectively adsorbed (Figure 3b). These findings agree well with other studies on hardwood Spodosols, Ultisols, and Inceptisols (Cronan 1990; Cronan & Aiken 1985; Guggenberger & Zech 1993; Jardine et al. 1989; Vance & David 1989), but differ from a spruce-fir Alfisol study (Donald et al. 1993).

Initial mass isotherm parameters were calculated from adsorption characteristics on the basis of both total DOC and DOC fractions. The spruce-fir Bhs horizon used in this study had higher slope (m), Kd, and RSP values than B horizons used in other studies, suggesting our Bhs soil has a greater affinity for DOC and a higher intrinsic DOC release rate (Table 3). The relatively large concentration of DOC determined in our study explains why the greatest RSP value was obtained. For the spruce-fir Bhs horizon, hydrophobic acids had the largest m, Kd, and RSP values as compared to other DOC fractions. Based on m and Kd values, hydrophobic acids were selectively adsorbed. The greatest RSP value was due to the largest proportion of this DOC component

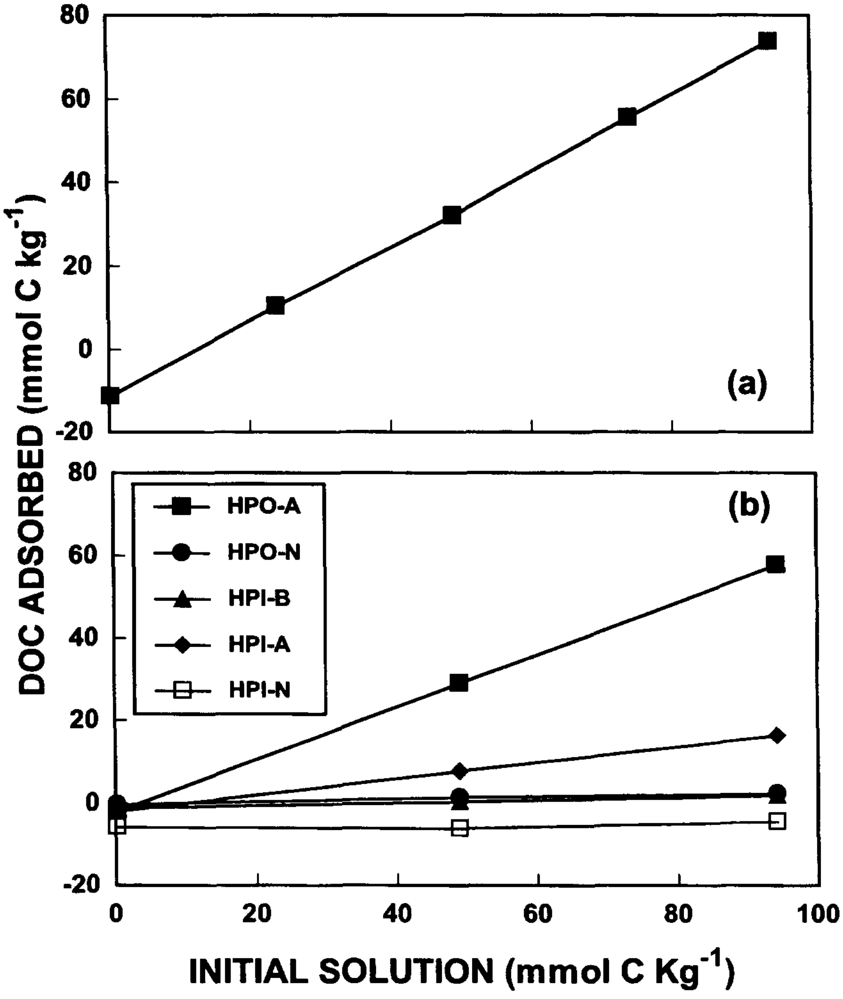


Figure 3. Initial mass isotherm adsorption of (a) bulk DOC and (b) DOC fractions by a Bhs horizon.

in the added solutions. Hydrophilic neutrals had a higher b value than the other DOC fractions, indicating hydrophilic neutrals make up the largest proportion of the organic C remaining in solution. The predominant mechanism of DOC and hydrophobic acids retention by mineral soils has been attributed to physical adsorption (Jardine et al. 1989). In our soils, however, ligand- and anion-exchange, and cation-bridging mechanisms can not be entirely ruled out (Parfitt et al. 1977). In contrast, Donald et al. (1993) demonstrated a near-equal sorption of hydrophobic and hydrophilic acids in their Bt horizon and attributed their findings to the different chemical and mineralogical properties

Table 3. Comparison of initial mass adsorption parameters among Maine spruce-fir Spodosol, Maine hardwood Spodosol (Vance & David 1989, 1992), New Hampshire hardwood Spodosol (Nodvin et al. 1986a), northeastern Bavaria Norway spruce Spodosol and Inceptisol (David & Zech 1990), and Saskatchewan spruce-fir Alfisol (Donald et al. 1993).

Horizon	Fraction	m	Kd·10^{-4} m^3kg^{-1}	RSP	–b
				- - - mmol C kg^{-1} - - -	
		Maine spruce-fir Spodosol			
Bhs	Total DOC	0.90	924	117.5	11.5
	HPO-A	0.64	175	6.2	2.2
	HPI-A	0.19	24	2.4	1.9
	HPI-B	0.03	3.3	1.3	0.2
	HPO-N	0.03	2.8	0.2	0.2
	HPI-N	0.01	1.4	6.2	6.1
		Maine hardwood Spodosol			
B	Total DOC	0.55	123	4.5	2.0
	HPO-A	0.42	70	3.5	2.0
	HPI-A	0.09	10	2.1	1.8
	HPO-N	0.02	2.0	0.1	0.1
	HPI-N	0.01	0.6	0.2	0.2
		Maine hardwood Spodosol			
Bhs	Total DOC	0.85	567	30.1	4.5
		New Hampshire hardwood Spodosol			
B	Total DOC	0.60	150	6.2	2.5
		NE-Bavaria Norway spruce Spodosol			
Bs	Total DOC	0.76	325	6.1	1.4
		NE-Bavaria Norway spruce Inceptisol			
Bs	Total DOC	0.83	488	58	9.9
		Saskatchewan spruce-fir Alfisol			
Bt	Total DOC	0.54	116	30.7	14.2

between Spodosols and Alfisols. Additional studies are required in order to delineate the adsorptive characteristics between hydrophobic and hydrophilic acids in a variety of ecosystems.

Table 4. Major chemical shifts in ^{13}C-NMR spectra of humic substances (see text for references).

Chemical Shift (ppm)	Type of Carbon
0–50	Alkyl C
50–60	Methoxy C
60–65	Alcohol C
65–95	Carbohydrate C and C-O or C-N Esters
95–110	Acetal C
110–160	Aromatic and Olefinic C
160–170	Phenolic C
170–185	Carboxyl, Ester, and Amide C
185–220	Aldehyde and Ketone C, and C = S
[(110–160) ppm/(0–220) ppm]* 100	Aromaticity (fa_1), %
[(110–160) ppm/(0–160) ppm]* 100	Aromaticity (fa_2), %
(0–50) ppm/(65–95) ppm	Alkyl/Carbohydrates Ratio

^{13}C NMR characterization of soils, bulk leachates, and DOC fractions

Table 4 lists the chemical shifts and type of C information determined from ^{13}C NMR analysis that were used to interpret C composition of samples evaluated in this study (Malcolm 1989; Wershaw 1985; Wilson 1987). Because of the possible overlap between different C functional groups, these divisions should be regarded only for comparative purposes. CP/MAS ^{13}C NMR spectra of the samples from different horizons in profile 1 are shown in Figure 4. The organic (Oi, Oe, and Oa) horizons had strong signals for lignin as suggested by chemical shift of 54, 114, 130, and 154 ppm (Hatcher 1987; Kögel et al. 1988; Kögel-Knabner 1992, 1993). Signals due to polysaccharides (cellulose and hemicellulose) were also found at 86 ppm and shoulders around 103 ppm in the organic horizons. In the alkyl region, signals at 36 ppm (organic horizons) and 29 ppm (mineral horizon) were assigned to C in long chain paraffin carbon in fatty acids and waxes (Kögel-Knabner 1992), in particular, carbon of polymethylene groups (Duncan 1987; Wilson et al. 1983). There were partially resolved signals around 11 and 4 ppm in the spectra of the three organic horizons. The signal at 11 ppm has been assigned to methyl groups terminal to alkyl chains (Preston & Schnitzer 1984). Broad signals centered around 175 ppm in the spectrum of the Bhs horizon arise from carboxyl, ester, and/or amide C (Wershaw 1985).

Integration of the major regions of ^{13}C resonances indicated the C components were mainly alkyl, carbohydrate, and aromatic for both organic and mineral soils (Figure 5). For all profiles examined, the signal intensities for

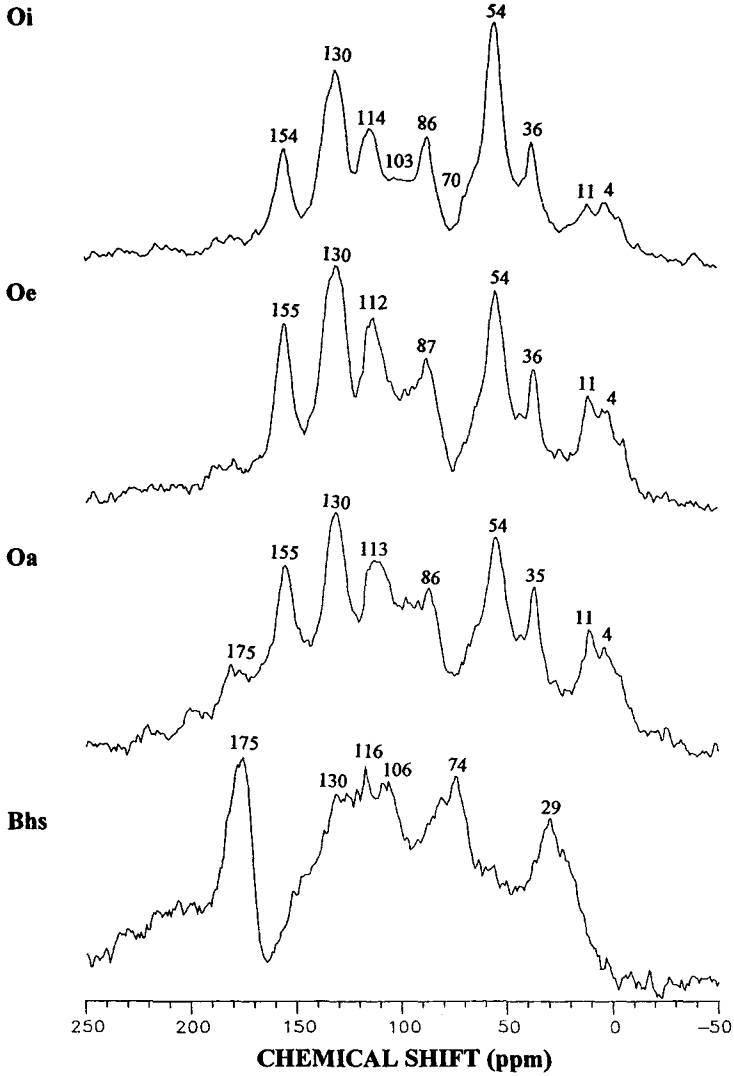


Figure 4. ^{13}C CP/MAS NMR spectra of horizons from profile 1.

alkyl and carboxylic C increased with depth. The increases in alkyl and carboxylic C with depth were the result of biodegradation of forest litter and oxidation of lignin side chains, respectively, that have been reported in several studies (Kögel-Knabner et al. 1988; Kögel-Knabner 1992; Zech et al. 1985). On the other hand, methoxy and carbohydrate C decreased with depth in all the profiles. The rapid removal of methoxy groups is known to

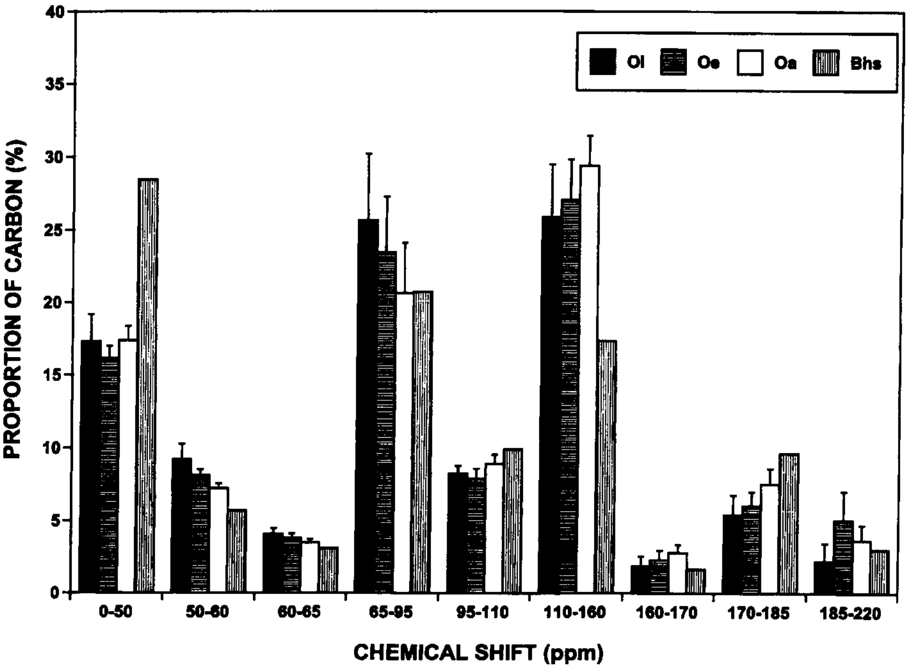


Figure 5. ^{13}C NMR composition of soil profiles collected from a spruce-fir forest. Values are means of five profiles except that only one sample was used for the Bhs horizon. Error bars represent the standard deviaton of the mean.

be one of the early steps in the decomposition of lignin (Oades et al. 1987). Preferential biological degradation has been suggested for the decrease in the carbohydrate C (Kögel-Knabner 1992, 1993). The increase of alkyl C and the decrease of carbohydrate C with increasing depth concurred with the findings by Gressel et al. (1996), who have shown similar trends with depth (from O to A horizons) while examining conifer forest Alfisol profiles. Decomposition increased with depth from the organic to the mineral horizons and resulted in a decrease of aromatic C (Figure 5; Table 5).

The composition of forest floor layers (O horizons) depends on inputs such as plant residues, microbial and animal products, and on the decomposition rate of different compound classes. Some C compositional changes in the forest floor layers were observed and these findings were comparable with several earlier studies on different hardwood and conifer forest soil types including Entisols, Histosols, Inceptisols, and Mollisols (Hempfling et al. 1987; Kögel-Knabner et al. 1988; Zech et al. 1990). Baldock & Preston (1995) suggested using the ratio of alkyl to carbohydrates C content to relate the extent of decomposition and C compositional changes in forest soil hori-

Table 5. The alkyl/carbohydrates ratio, aromaticity (fa_1 and fa_2) of soil materials collected from a spruce-fir forest. Values are means and standard errors (in parentheses) based on five soil profiles, except that only one sample was used for the Bhs horizon.

Type of Carbon	Horizon			
	Oi	Oe	Oa	Bhs
	%			
Aromaticity (fa_1)	25.9 (3.6)	27.1 (2.8)	29.9 (1.8)	17.4
Aromaticity (fa_2)	28.7 (4.2)	31.5 (3.6)	34.4 (2.1)	20.3
Alkyl/Carbohydrates Ratio	0.8 (0.2)	0.8 (0.2)	1.0 (0.2)	1.4

zons. Both aromaticities and alkyl/carbohydrates ratios of the three organic horizons indicated that the changes were minimal (Table 5). The aromatic C proportion remained relatively constant, suggesting similar decomposition rates for aromatics and total organic C, or an equilibrium between decomposition and inputs of root litter (Zech et al. 1990). Overall, our ^{13}C-NMR results suggested the acidic soil environment in this spruce-fir forest caused decomposition and humification in the forest floor to proceed very slowly.

There were considerable differences, however, in the C composition among bulk leachates and DOC fractions (Figure 6). The NMR data suggested that the C composition of bulk leachates and DOC fractions was primarily alkyl, carbohydrate, aromatic, and carboxylic C (Figure 7). Hydrophobic acids had a slightly larger proportion of alkyl C than bulk leachates and other DOC fractions (Figure 7). Hydrophobic acids also had the largest aromatic and the smallest carbohydrate contents (Figure 7; Table 6). Hydrophilic acids had the highest proportion of C as carboxylic groups, suggesting a high degree of microbial oxidation of the hydrophilic acids with respect to other organic materials (Guggenberger et al. 1994). A high percentage of carboxylic C in hydrophilic acids is consistent with other studies (Guggenberger et al. 1994; Vance & David 1991b). Guggenberger et al. (1994) have suggested that hydrophilic acids represent substances that are, at least partially intermediates in the hydrophobic acid decomposition process. Hydrophilic neutrals had distinctive signals in the alkyl (0–50 ppm) region and were also richer in carbohydrate C as compared to other organic materials (Figure 7). We also observed relatively high content of aromatic C in the hydrophilic neutrals as compared with the study of Guggenberger et al. (1994). In their study, a cation exchange resin (Duolite A-7) was used to retain the hydrophilic acids, which in turn, resulted in a lower proportion of hydrophilic neutrals. Accordingly, the reduced efficiency of the XAD-4 resin could have allowed some aromatic material to appear in our hydrophilic neutral fractions.

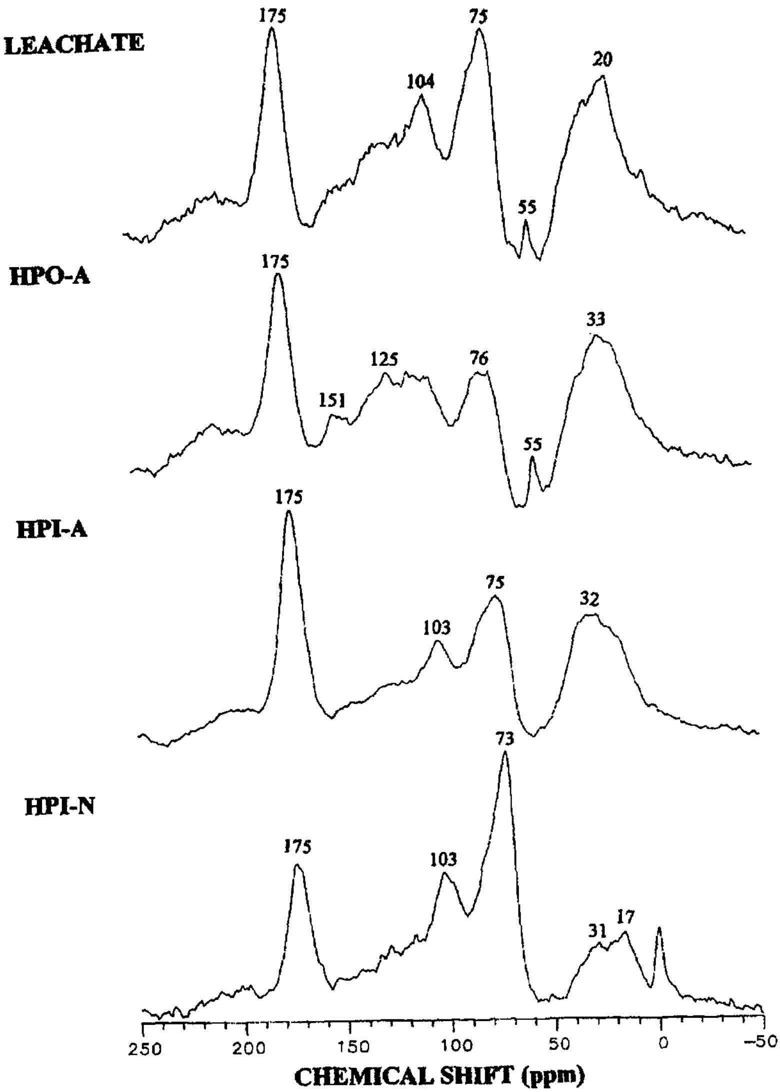


Figure 6. ^{13}C CP/MAS NMR spectra of forest floor bulk leachates, hydrophobic acids (HPO-A), hydrophilic acids (HPI-A), and hydrophilic neutrals (HPI-N).

Hypothetically, the bulk sample contains characteristics of the indigenous C material, and in order to further evaluate the impact due to the isolation process on the C types present in the DOC fractions to the bulk sample, a methodological test was initiated as follows. The amount of carbon in each chemical-shift region of the spectra of each DOC fraction was multiplied by

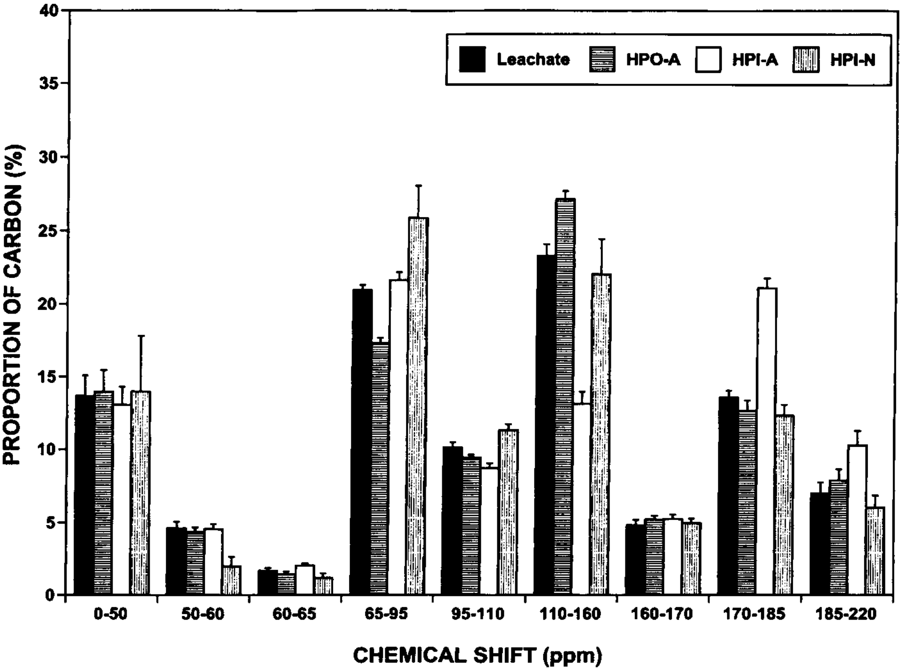


Figure 7. Carbon composition as determined by ^{13}C NMR of forest floor bulk leachates, hydrophobic acids (HPO-A), hydrophilic acids (HPI-A), and hydrophilic neutrals (HPI-N) collected from a spruce-fir forest over two years. Values are means based on 16,18, 14, and 4 samples in leachates, HPO-A, HPI-A, and HPI-N, respectively. Error bars represent the standard deviation of the mean.

Table 6. The alkyl/carbohydrates ratio, aromaticity (fa_1 and fa_2) of 16 leachates, 18 HPO-A, 14 HPI-A, and 4 HPI-N collected from June, 1992 to November, 1993. Values are means and standard errors (in parentheses).

Type of Carbon	Leachate	HPO-A	HPI-A	HPI-N
	%			
Aromaticity (fa_1)	23.3 (0.8)	27.2 (0.5)	13.2 (0.8)	22.0 (2.4)
Aromaticity (fa_2)	31.2 (1.0)	36.7 (0.8)	20.6 (1.0)	29.0 (3.7)
Alkyl/Carbohydrates Ratio	0.7 (0.2)	0.8 (0.1)	0.6 (0.1)	0.5 (0.1)

its percent yield from the fractionation analysis. The contribution of each individual DOC fraction was summed and denoted as a model value using the following equation:

$$\text{Model (\%)} = [(F_{\text{HPO-A}})(P_{\text{HPO-A}}) + (F_{\text{HPI-A}})(P_{\text{HPI-A}}) + (F_{\text{HPI-N}})(P_{\text{HPI-N}})] \quad [4]$$

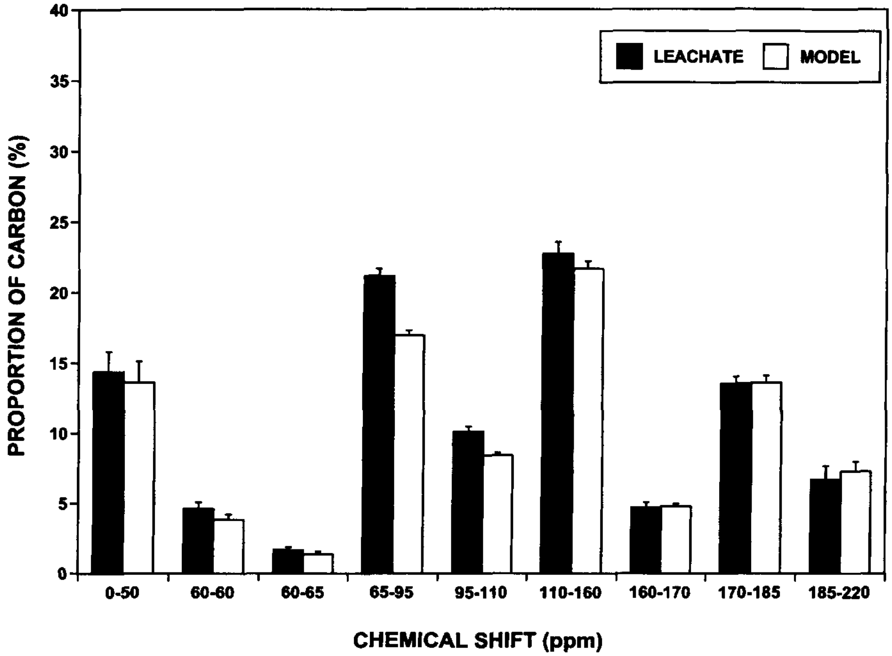


Figure 8. ^{13}C NMR composition of forest floor bulk samples and values calculated from the model (see Eq. [4] in the text for detailed calculation) between 1992 and 1993.

where F and P represent the DOC fraction and percent C from NMR for the hydrophobic acids, hydrophilic acids, or hydrophilic neutrals, respectively. The relative proportions of different C forms measured in bulk leachate samples between 1992 and 1993 were rather close to the values calculated from Eq. [4] (Figure 8). This is strong support that indicates the DOC fractions are unaltered during the isolation process since the bulk sample was never isolated and was minimally processed.

Relationships between soil and soil solution C

In order to examine how C composition changed after the forest floor leachates contacted the Oa horizon, different C classes were compared between the Oa horizon and the bulk leachates (Figures 5 and 7). Results indicated the phenolic (160–170 ppm), carboxylic (170–185 ppm), and carbonyl C (185–220 ppm) appeared to dissolve easily, whereas alkyl (0–50 ppm), methoxy (50–60 ppm), alcohol (60–65 ppm), and aromatic C (110–160 ppm) did not seem to readily dissolve (Figures 5 and 7). The carboxylic C is probably the major contributor to organic acidity in these organic dominated leachates. Similarly, the C composition in the Bhs horizon, DOC, hydrophobic and hydrophilic acids suggested that DOC materials adsorbed on the Bhs horizon appeared to undergo further decomposition. This inference was based on the C compositional changes that occurred in the Bhs horizon as denoted by the increase in alkyl C (Figures 5 and 7). On the other hand, due to the lack of a decrease in methoxy C and an increase in carboxylic C in the Bhs horizon, lignin decomposition was the same either in an adsorbed or solution state. Several explanations are possible for the lack of further lignin degradation observed in this Bhs horizon. One possibility would be that the microbial community in this acidic (pH $\approx$ 4.8, Table 1) Bhs horizon is probably not dominated by white-rot fungi based on the fact that lignin biodegradation is attributed mainly to white-rot fungi (Baldock & Preston 1995).

^{13}C NMR duplicate analysis

Following the DOC duplicate isolation analysis, the two individual isolated DOC fractions (hydrophobic acids and hydrophilic neutrals) were examined by ^{13}C NMR and were found to have a nearly identical C composition (Figure 9). The NMR results are further support that the isolation scheme used in this study is remarkably reproducible with respect to C composition, results not previously documented.

^{13}C NMR annual patterns for bulk leachates and DOC fractions

For all the organic materials examined over the two-year study period (except hydrophilic neutrals that were only collected in 1992), we observed several trends with respect to compositional changes in the organic materials (Figure 10). Alkyl C decreased during 1992 but remained relatively constant in 1993, methoxy and alcohol C increased in 1992 but stabilized thereafter, carbohydrate and acetal C were relatively stable, and carboxylic and carbonyl C increased throughout the two-year study period.

The alkyl signal intensity has been separated into two components, i.e., mobile and rigid alkyl structures based on a study of the ability of microorganisms to synthesize alkyl C structures using a dipolar dephasing ^{13}C NMR technique (Kögel-Knabner et al. 1992). The decrease of alkyl C in 1992 may suggest that mobile structures were preferentially decomposed. The increase in alkyl C in June of 1993 and its subsequent stabilization suggested that rigid alkyl C could contribute to the accumulation of alkyl C. The relatively stable proportions of carbohydrate C during 1992 and 1993 suggested that white-rot fungi were probably important microbial species in this forest floor community based on the report that white-rot fungi has been shown not to preferentially decompose carbohydrate C (Kirk & Highley 1973). The accu-

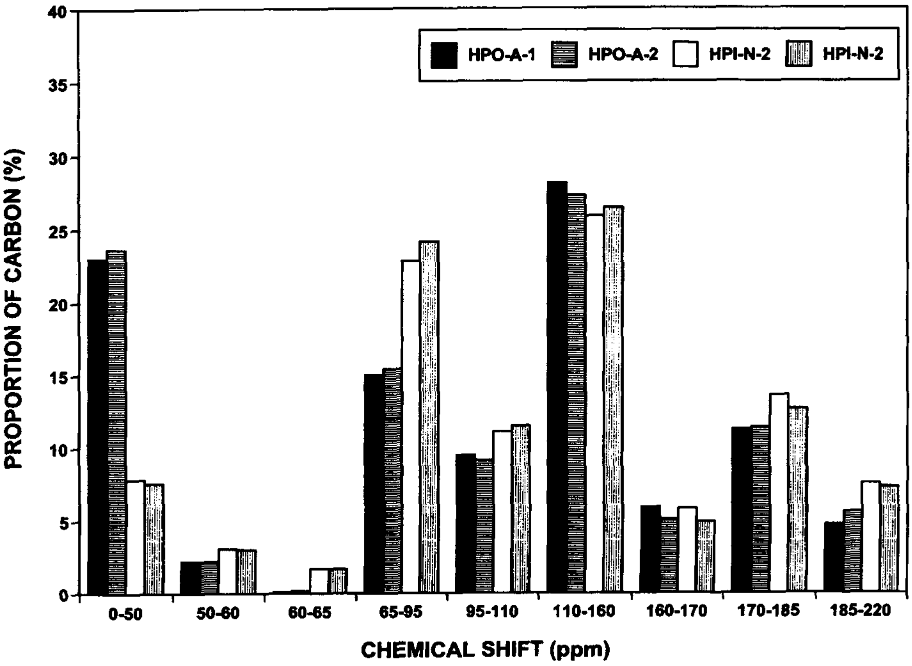


Figure 9. ^{13}C NMR composition of hydrophobic acids replicate 1 (HPO-A-1), hydrophobic acids replicate 2 (HPO-A-2), hydrophilic neutrals replicate 1 (HPI-N-1), and hydrophilic neutrals replicate 2 (HPI-N-2) collected from a spruce-fir forest in August 1992.

mulation of carboxylic C suggested that oxidative decomposition processes were probably not limited in this ecosystem.

Conclusion

In the Howland spruce-fir ecosystem, concentrations of DOC in bulk leachates collected over two years followed a distinct seasonal pattern, with greatest concentrations in September of 1992 and 1993. In all bulk leachate solutions, hydrophobic and hydrophilic acids dominated DOC fractions. Consequently, this suggested that these acid DOC fractions will exert an important influence on the solution chemistry of forest soils.

The adsorption of bulk DOC and preferential adsorption of hydrophobic acids by the spodic B horizon confirmed several previous reports. Based on these investigations, the modification of DOC concentrations and constituents by mineral soil adsorption is highly relevant to subsurface and aquatic environments. In particular, the hydrophobic to hydrophilic acid ratio may be a good indicator of the contact of forest floor leachates with the B horizon at

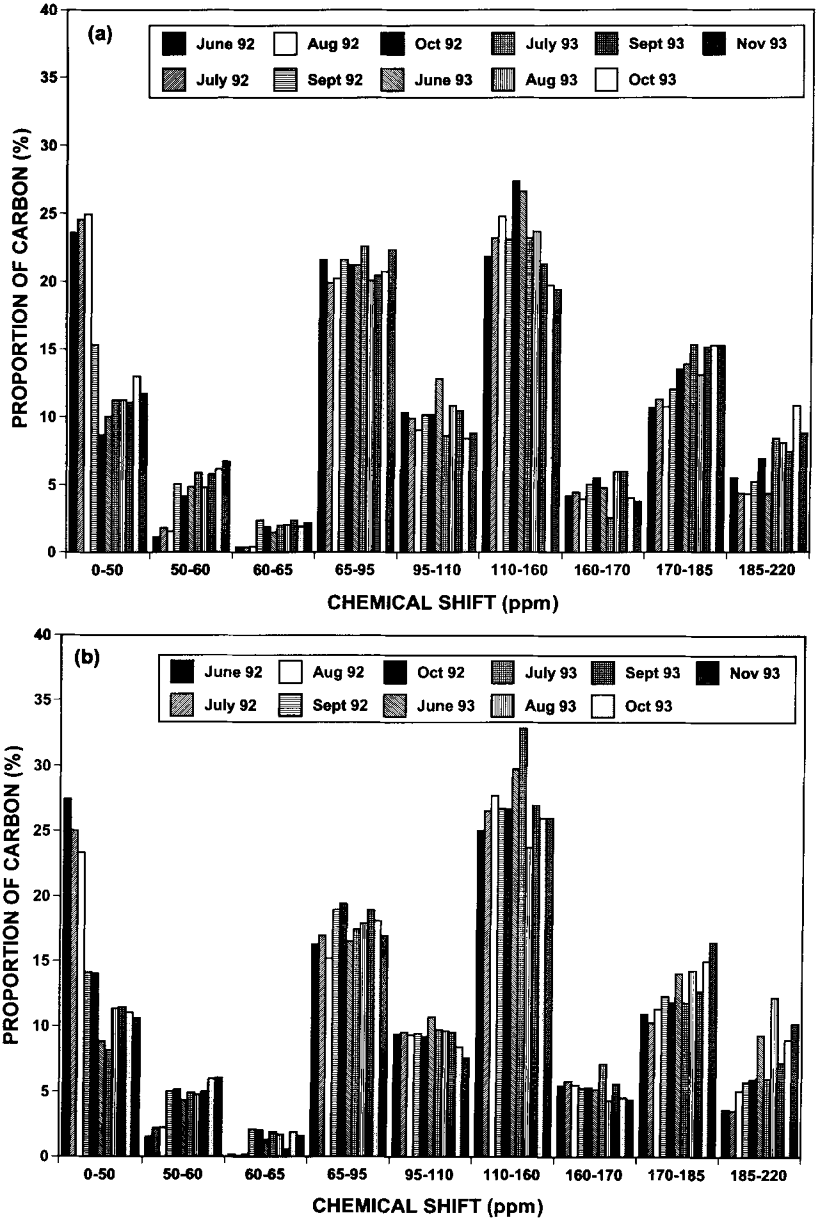
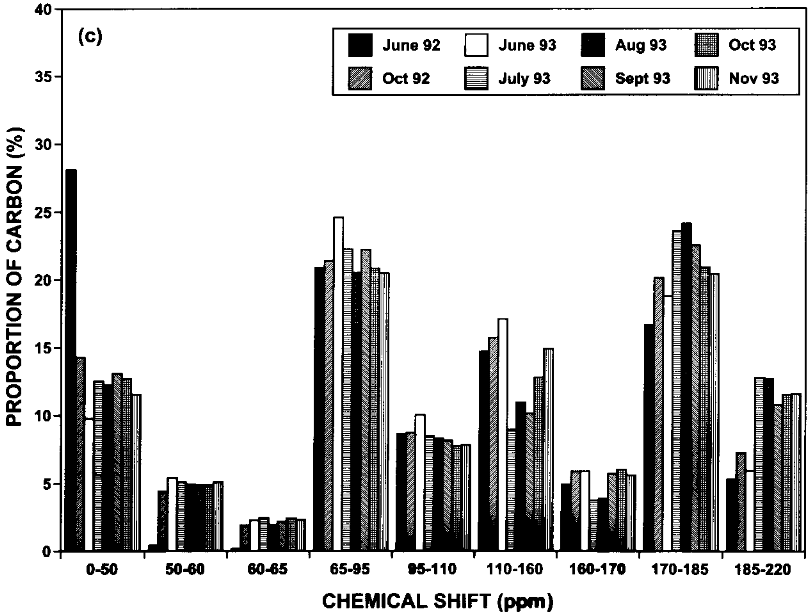


Figure 10. Carbon composition as determined by ^{13}C NMR of (a) forest floor bulk leachates, (b) hydrophobic acids, (c) hydrophilic acids, and (d) hydrophilic neutrals collected in a spruce-fir forest from June 1992 to November 1993.



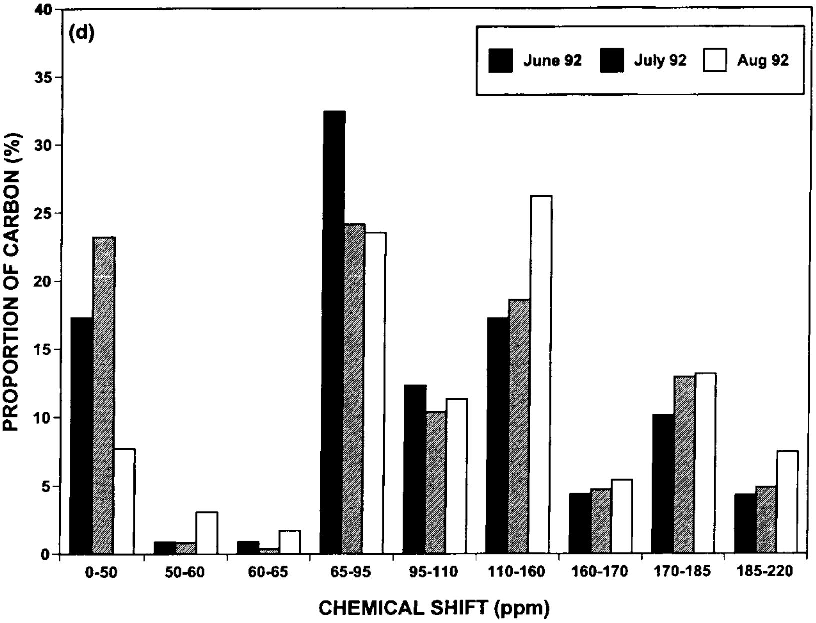


Figure 10. Continued.

least for Spodosols, Ultisols, and Inceptisols. This is important since the composition of DOC affects cation leaching, acid-base chemistry of soil solutions and surface waters (Cronan & Aiken 1985; David & Vance 1991; McKnight et al. 1985; Pohlman & McColl 1988; Vance & David 1989).

CP/MAS ^{13}C NMR data suggested alkyl, carbohydrate, and aromatic C were the primary C constituents for organic and mineral soils. An increasing proportion of alkyl C with horizon depth implies biodegradation has occurred. On the other hand, with increasing depth and decomposition in the soil, the proportion of methoxy, carbohydrate, and aromatic C decreased. The C forms in the soil profile are primarily determined by source inputs and decomposition processes. Our findings indicated that decomposition rates of different C compounds were relatively slow in this spruce-fir soil.

^{13}C NMR analysis also indicated that alkyl, carbohydrate, aromatic, and carboxylic C were the major constituents of bulk leachates and DOC fractions. The C composition among bulk leachates and DOC fractions was quite variable. Hydrophobic acids tended to be more aromatic than the other organic materials, whereas hydrophilic acids were highly carboxylic, and hydrophilic neutrals contained the largest proportion of carbohydrate groups. The C composition in the Oa horizon and the bulk leachates suggested that substances containing high contents of phenolic, carboxylic, and carbonyl groups dissolved readily. DOC materials adsorbed on the Bhs horizon underwent further decomposition as revealed by the C compositional changes among Bhs horizon, DOC and hydrophobic and hydrophilic acids. Distribution patterns of C composition were observed over the two-year study period. These observed annual patterns suggested that certain microorganisms, such as white-rot fungi, might be important in affecting the C composition of bulk leachates collected in the forest floor of this spruce-fir ecosystem.

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