^{13}C–NMR OF FOREST SOIL LIPIDS

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Molecular characterization of soil lipids often provides valuable biogeochemical information about the impact of vegetation, microorganisms, and abiotic factors on the soil C sequestration proccess. The total lipid extracted with petroleum ether from nine soils developed under three types of Mediterranean forest (stone pine (*Pinus pinea* L.), evergreen oak (*Quercus rotundifolia* L.), and Spanish juniper (*Juniperus thurifera* L)) has been analyzed by high-resolution ^{13}C nuclear magnetic resonance (^{13}C-NMR) under quantitative acquisition conditions. Tentative assignments of the spectral peaks are presented, and the spectra of soil lipids are compared with those from the lipids extracted directly from leaves of the corresponding trees. This comparison evidenced that soil lipids behaved as biomarker soil fractions when analyzed by ^{13}C-NMR as a whole. Analysis by gas chromatography-mass spectrometry (GC/MS) reveals that the volatile fraction of the lipid extract (46%, on average, as estimated by internal reference) consisted mainly of free alkanes, alkanoic acids ($<C_{30}$), and diterpene resin acids.

We observed some differences between the chemical structures suggested by ^{13}C-NMR and GC/MS. This was interpreted as a portion of soil and plant lipids consisting of extractable material that cannot be detected by standard GC methods. The complex signal pattern in the 0 to 30 ppm chemical shift range showed typical signals for carbons in acyl polymethylene chains, which overlapped with a pattern suggesting isoprenoid-like branching in long-chain structures (major signals at ca. 22, 26, and 32 ppm). In addition, periodic unsaturations suggested by signals at ca. 124 and 135 ppm are also compatible with polyprenoid-type backbones. The alkyl region coincided with those of mono- to triacyl glycerol fatty esters. It seems evident that ^{13}C-NMR allows us to characterize structures present in nonvolatile complex material. (Soil Science 2001;166:186–196)

Key words: Lipids, alkanes, fatty acids, resin acids, polyprenols, pine, oak, juniper.

THE lipid fraction, *i.e.*, the complex fraction soluble in low-polarity organic solvents, has been studied intensively in soils (Breger, 1966; Braids and Miller, 1975; Jambu et al., 1978; Andreyev et al., 1980; Stevenson, 1982). The reason for this interest is that the molecular composition of lipids is considered to be a source of ecological information about the structure of the trophic system (Philp, 1985) and an indicator of the performance of the biogeochemical cycle (Stevenson, 1982; Almendros et al., 1996). Extractable lipid accounts for a low portion of total soil C, but this fraction may have important direct and indirect effects on the physico-chemical and biological processes in soils: most lipids influence the surface properties of aggregates and, thus, soil structural stability (Jambu et al., 1983; Dinel et al., 1990) whereas other lipids show antimicrobial properties (Lynch et al., 1976).

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Few studies carried out on soil lipids have used nondestructive techniques such as ^{13}C-NMR spectroscopy (Schnitzer and Preston, 1987; Amblès et al., 1991; Pollesello et al., 1993; Gunstone, 1994); most of the report are of lipid composition as revealed by GC/MS. In the present study the molecular compositions of plant and soil lipids in forest ecosystems comprising typical continental Mediterranean forests of pine, oak, and juniper are analyzed by ^{13}C-NMR and GC/MS. The combination of these two sets of analytical data should provide a comprehensive description about the nature and evolution of the entire soil lipid fraction.

MATERIALS AND METHODS

Samples Studied

Nine soil samples were taken from widely different forest ecosystems, representative of undisturbed or reforested continental Mediterranean ecosystems, in Central Spain. The characteristics of the soils are given in Table 1 and have been reported in detail elsewhere (Almendros et al., 1996). The soils were developed under three types of vegetation: stone pine (umbrella pine: *Pinus pinea* L.), evergreen oak (holm oak: *Quercus rotundifolia* Lam.) and Spanish juniper (*Juniperus thurifera* L.).

Sampling took place in the spring and early summer. Soil samples were collected (organic horizons: O + A) with a spade after removal of the litter layer. The soil material was then air-dried, crushed with a wooden cylinder, and sieved to 2 mm. Plant and mineral fragments bigger than 2 mm were discarded.

The plant material was collected directly from the trees in the corresponding forests. The leaves were air-dried and homogenized to 3 mm with a rotary knife mill. Both soil and plant lipid samples were labeled P, O, and J, refering to the pine, oak, and juniper materials from which the materials were derived, respectively.

Extraction of the Lipid Fraction

Petroleum ether (40–70 °C) was used to remove the low polarity fraction with the intent of minimizing the extraction of oligomeric, colored humus fractions that could be dissolved by more polar, less selective solvents (*i.e.*, striking differences between GC/MS and ^{13}C-NMR results would be obvious from such a lipid fraction, assuming the low volatility of macromolecular materials). The extraction period, which lasted 3 days, used a 1-L Soxhlet containing an extraction thimble with ca. 300 g of soil. The solvent was changed every 12 hours. The extract was dehydrated with anhydrous Na_2SO_4, evaporated under reduced pressure to ca. 50 mL, dried under a N_2 stream at room temperature, and the residue was weighed. The soil lipids were a solid, pale yellow, waxy material, and the plant lipids had a dark green or brown color.

Preliminary Lipid Characterization by Gas Chromatography

The lipids were methylated with ethereal diazomethane and injected into a HP 5890 gas chromatograph coupled to an HP 5971A mass detector with a 25-m × 0.22-mm internal diameter, cross-linked, OV-1 capillary column. Helium flow was 1 mL min^{-1}. The oven temperature was set to 40 °C during the splitless period, then raised to 100 °C (32 °C min^{-1}). The temperature was programmed from 100 °C to 270 °C (rate = 6 °C min^{-1}) during the chromatographic run. The electron impact mass spectra were acquired at 70 eV.

Quantitative chromatographic analyses of petroleum ether extracts were carried out by gas chromatography with a flame ionization detector using ethylvanillin as the internal standard. On average, about 46% of the sample material appears in the chromatographic profile.

Nuclear Magnetic Resonance Spectrometry

High-resolution ^{13}C-NMR spectra (75.4 MHz, $CDCl_3$) were obtained with a Bruker MSL 300 in a 10-mm multinuclear probe head in the deuterium lock mode. The spectra were referenced to a coaxial capillary tube with tetramethylsilane. No attempt was made to correct magnetic susceptibility. Spectra were acquired with inverse gated broadband proton decoupling. The pulse sequence used (Ring Down Elimination program; Gerothanassis, 1986) consisted of:

$$90^\circ_{+x} - \Delta t - \mathrm{FID}^{(+)} - T_d - 90^\circ_{-x} - \Delta t - \mathrm{FID}^{(-)} - T_d - 180^\circ_{+y} - 90^\circ_{-x} - \Delta t - \mathrm{FID}^{(+)} - T_d - 180^\circ_{+y} - 90^\circ_{+x} - \Delta t - \mathrm{FID}^{(-)} - T_d$$

A total of 30,000 free induction decays were accumulated with an acquisition period of 0.16 s. The T_d was set to 0.5 s and Δt to 20 μs. Under the above conditions, the chemical shifts given are considered reliable by ±1 ppm. The areas under different spectral regions were computed by using the integration routine of the software supplied with the instrument. The four major chemical shift ranges considered for integration were: 0–46

TABLE 1

Analytical characteristics of soil samples (0–10 cm depth)

Sample	Soil type/humus type	Altitude m	Slope %	Sand g kg^{-1}	Silt g kg^{-1}	Clay g kg^{-1}	pH (H_2O)	CEC mmol$_c$ kg^{-1}	C g kg^{-1}	Lipid g kg^{-1}	C/N	CEC/C	S mmol$_c$ kg^{-1}	S/CEC
P1	Cambisol/Xeromor	710	15	831	113	56	5.4	360	100	1.5	30	3.6	155	0.43
P2	Regosol/Sandy moder	765	0	935	27	38	6.1	120	33	0.6	20	3.6	48	0.40
P3	Cambisol/Xeromor	835	0	912	30	58	6.2	255	98	2.1	26	2.6	181	0.71
O1	Cambisol/Mesotrophic mull	670	2	716	149	135	5.6	185	47	0.2	17	3.9	97	0.52
O2	Cambisol/Sandy moder	850	0	828	70	102	6.6	485	127	1.4	21	3.8	437	0.90
O3	Cambisol/Sandy moder	1010	15	378	488	134	6.4	290	90	1.1	24	3.2	245	0.84
J1	Cambisol/Sandy moder	1195	15	637	167	196	7.0	397	140	1.0	21	2.8	285	0.72
J3	Cambisol/Calcic mor	1020	15	300	580	120	7.5	597	215	4.0	27	2.8	596	1.00
J2	Leptosol/Eutrophic mull	1200	0	392	384	224	7.6	522	73	0.6	14	7.1	397	0.76

S = sum of exchangeable bases (Na + K + Ca + Mg).

Sample labels (P = pine forest, O = oak forest, J = juniper forest) refer to Materials and Methods section.

ppm = alkyl; 46–110 ppm = O-alkyl; 110–160 ppm = aromatic/unsaturated; and 160–200 ppm = carbonyl (Wilson, 1987).

Statistical Treatments

Attempts to identify some different tendencies in the spectroscopic quantitative patterns in the plant species were carried out by discriminant analysis. The original data matrix consisted of the signal area values of the most prominent peaks in the spectra (the variables) from the plant and soil lipids (the individuals). When using the automatic backwards variable selection option, this treatment (ITCF, 1988) extracts the significant independent variables most useful for discriminating between the lipid samples previously classified into three supervised sets (pine, oak, juniper) and yields discriminant functions (linear combination of the original variables) that are useful for graphical representation, in a reduced-dimension space, of the similarities between the individuals under analysis. This is useful both to reveal any chemotaxonomic potential of the ^{13}C-NMR patterns in terms of vegetation type and to select objectively the most diagnostic NMR signals for every vegetation type. This method also indicates the extent to which the samples are properly classified into the sets originally defined and yields the coefficients of the original variables in the discriminant functions.

RESULTS

Gas Chromatographic-Mass Spectrometric Analyses

The major constituents of the extractable lipids (Tables 2 and 3) were *n*-fatty acids (which represent between 4 and 54% of the total volatile compounds) and alkanes (up to 37%).

The lipids from the O series did not yield any compounds other than the above-mentioned major constituents, whereas lipids from gymnosperms (P series, J series) yielded appreciable amounts of resin acids and cyclic hydrocarbons (up to 65.4%), the most abundant structures of which are shown in Table 2. Abietanes were more abundant in samples from the P series than from J series, whereas the opposite occurred with pimaranes. In general, and when compared with abietanes, labdanes (characteristic of juniper vegetation), and to a lesser extent pimaranes, are present in lower concentrations in soil samples than in leaf samples (Table 2).

^{13}C-NMR Spectra

Figures 1, 2 and 3 show the spectral ranges most crowded with signals of the ^{13}C-NMR

TABLE 2

Major volatile compounds[a] in methylated lipid fractions from plant and soil material from Mediterranean forests of Central Spain

	Plant lipids	Soil lipids			Plant lipids	Soil lipids			Plant lipids	Soil lipids		
	P	P1	P2	P3	O	O1	O2	O3	J	J1	J2	J3
C_2-Alkylnaphthalenes	—	—	—	—	—	—	—	—	—	—	—	3.0
δ-Cadinene	—	0.5	0.7	0.3	—	—	—	—	0.7	0.1	4.5	—
Totarol	—	—	—	—	—	—	—	—	—	—	9.7	—
Manoyloxide	—	—	0.6	—	—	—	—	—	1.0	0.7	—	2.1
Methyl 8,11,13-abietatrien-18-oate [dehydroabietate]	23.0	12.0	25.0	12.0	—	4.1	—	—	4.6	0.4	5.2	0.4
14-Isopropyl-13-methoxy-podocarpa-8,11,13-trien-3-one	—	—	—	—	—	—	—	—	—	0.1	1.1	1.9
Methyl 7,13-abietadien-18-oate [abietate]	14.0	6.9	—	2.5	—	—	—	—	2.5	0.4	—	—
Methyl 7,15-isopimaradien-18-oate [isopimarate]	—	1.6	3.4	3.2	—	0.4	—	—	3.0	2.0	1.5	6.9
Methyl 8(14), 15-isopimaradien-18-oate [sandaracopimarate]	1.5	2.8	—	—	—	—	2.6	4.1	2.0	2.4	1.5	—
Methyl 8(17), 12,14-labdatrien-19-oate [communate]	—	—	—	—	—	—	—	—	7.4	—	—	—
Methyl 8(14), 15-pimaradien-18-oate [pimarate]	6.4	11.0	9.0	1.9	—	—	—	—	0.5	0.7	6.2	—
Methyl 2β-[2′ (*m*-isopropylphenyl)ethyl]-1β, 3α-dimethyl-cyclohexanecarboxylate [secodehydroabietate]	3.0	6.1	7.8	—	—	—	—	—	—	—	—	—
Methyl 7-oxodehydroabietate	—	—	—	—	—	—	—	—	—	1.3	2.1	—
Methyl 7-hydroxydehydroabietate	2.3	2.3	2.8	—	—	—	—	—	—	1.3	0.8	—
Methyl 15-hydroxydehydroabietate	0.6	—	1.2	—	—	—	0.1	—	—	4.7	3.8	—

[a]Representing more than 2% of the total chromatographic area in at least one of the 12 samples studied. Compounds representing less than 0.1% (dashes) were not taken into account. Sample labels refer to Table 1.

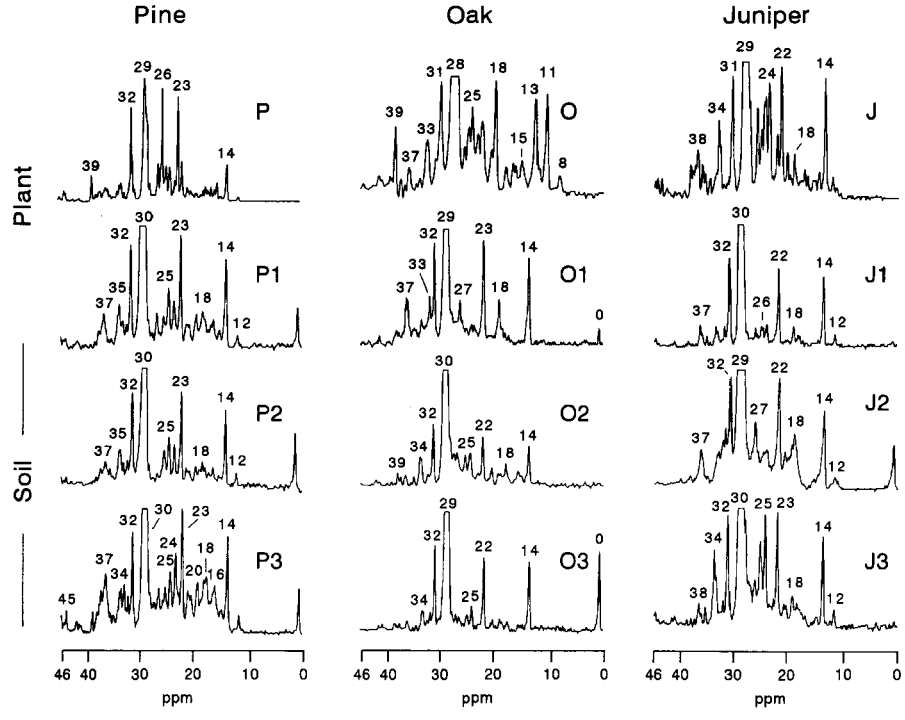

Fig. 1. Details of the alkyl region of the ^{13}C-NMR spectra of soil and plant lipid from continental Mediterranean forests.

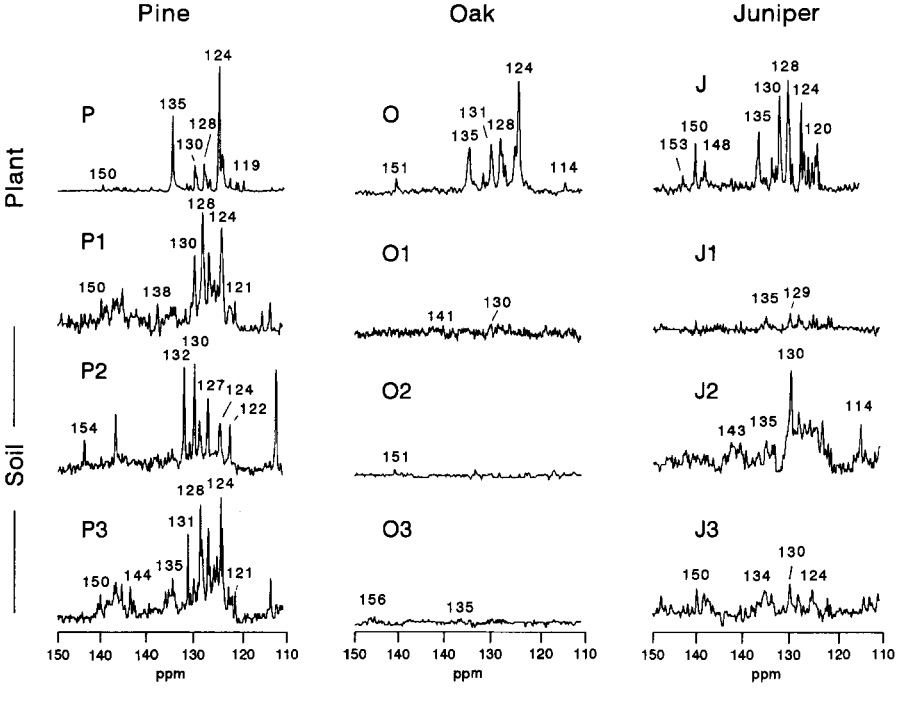

Fig. 2. Details of the unsaturated/aromatic region of the ^{13}C-NMR spectra of soil and plant lipid from continental Mediterranean forests.

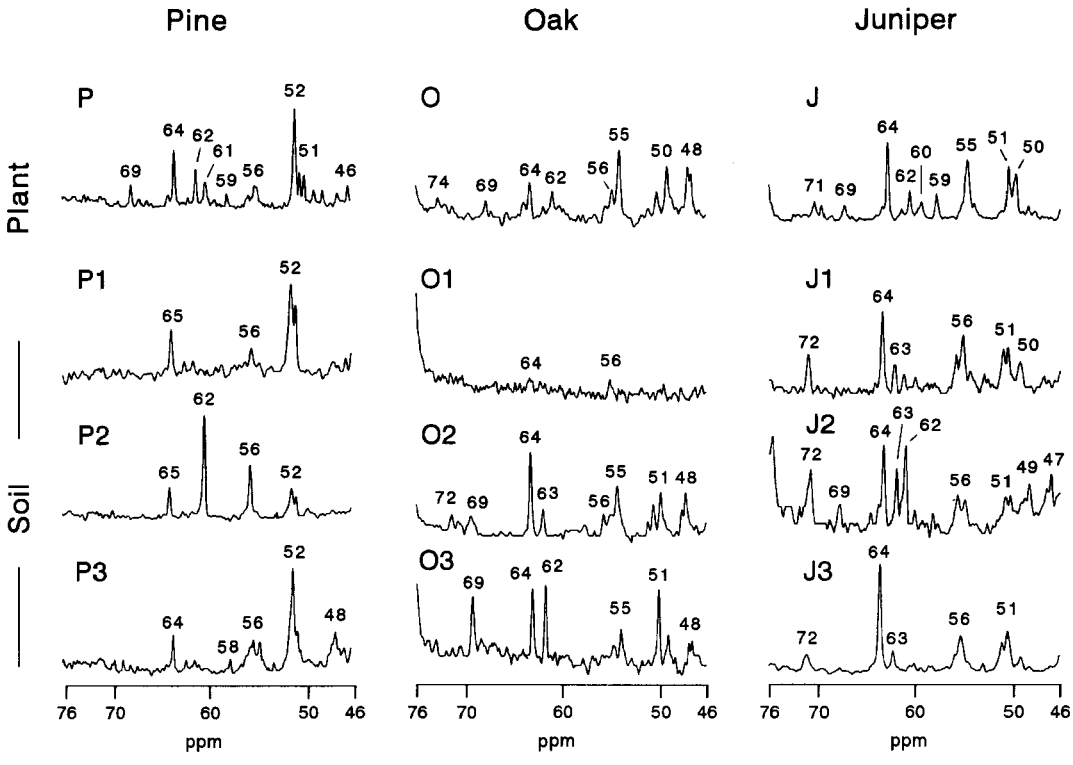

Fig. 3. Details of the O-alkyl region of the ^{13}C-NMR spectra of soil and plant lipid from continental Mediterranean forests.

spectra of the plant and soil extracts studied. As expected from the data in Table 2, most signal intensity is found in the aliphatic range from 0 to 46 ppm. Nevertheless, signals are also observed in the O-alkyl region (46–110 ppm), which includes weak resonances in the range between 46 and 50 ppm, typical for saturated homocyclic ring systems. The range between 110 and 160 ppm is assigned to aromatic and olefinic carbons. Between 160 and 200 ppm, carboxyl and carbonyl structures are found. The tentative assignations of the signals corresponding to distinct C types are compiled in Table 4.

Apart from the major peak at 29 ppm expected for straight-chain alkane carbons, the large number of distinct peaks in the (0–46 ppm) alkyl region (Fig. 1) suggests a substantial proportion of highly branched and/or cyclic structures. At first sight, these signals would not be assigned to diterpene hydrocarbons, in particular because they are also well defined in soils under oak, where resin acids are missing in the chromatogram (Tables 2 and 3). However, despite the major peak of ca. 29 ppm, which suggests the dominance of carbons in long polymethylene chains, there was a characteristic peak at 18 ppm in addition to other more or less constant peaks centered at ca. 23, 26, 32, and 38 ppm, which showed some systematic differences in terms of vegetation. In particular these peaks coincided with the chemical shifts of isoprenoid-type structures, with the possible inclusion of periodic in-chain unsaturations. Of the natural compounds showing such a structure, the polyprenols (up to 30 isoprene units) have been studied most extensively (Sasak and Chojnaki, 1973; Suga et al., 1989). It has also been observed that most peaks in the alkyl region (Table 4) coincided with those of the major C-types in the repeated isoprene units, *i.e.*, the major signals at 23 (C_5-*cis*), and ca. 38 ppm (C_1-*trans*), ca. 27 (C_4), and 18 ppm (ω5) (Chojnacki et al., 1987; Swiezewska and Chojnacki, 1988).

Other peaks in the 14 to 35 chemical shift range coincided with those reported for the carbons in extreme positions of fatty acid chains: Cα produced a signal at ca. 34 ppm and Cβ at ca. 24 ppm. The resonances from subsequent carbons in acyl alphatic chains (Table 4) show a very similar chemical shift, contributing to the large 29-ppm signal. The carbons on the terminal side of the

TABLE 3

Relative concentrations[a] of the major compound groups in lipid fractions from plant and soil material from Mediterranean forests of Central Spain

	Plant lipids	Soil lipids			Plant lipids	Soil lipids			Plant lipids	Soil lipids		
	P	P1	P2	P3	O	O1	O2	O3	J	J1	J2	J3
Total monoterpenes	—	0.3	—	2.6	—	—	—	—	—	0.7	—	—
Total sesquiterpenes	0.5	0.5	0.7	1.1	—	0.1	—	—	4.9	0.8	7.0	0.7
Total diterpenes	65.4	37.0	57.6	54.5	—	6.8	—	—	61.9	6.9	24.0	26.7
Total abietanes	44.7	26.9	39.7	41.0	—	4.1	—	—	7.1	1.6	18.3	11.2
Total pimaranes	20.7	10.1	17.2	12.2	—	2.7	—	—	43.0	4.6	5.7	10.0
Total labdanes	—	—	0.7	1.3	—	—	—	—	11.8	0.7	—	5.5
Total non-terpenic decalins	—	1.2	—	1.5	—	0.6	—	—	—	3.4	—	—
Total non-terpenic naphthalenes	—	—	—	0.8	—	0.9	—	—	—	—	—	—
Total hydrocarbons (other than *n*-alkanes)	0.5	1.9	1.8	9.7	—	1.6	—	—	1.5	4.9	5.2	0.6
Total alicyclic (no aromatic ring)	35.2	18.7	18.6	20.7	—	3.4	—	—	62.2	10.2	12.5	15.8
Total aromatics (at least one ring)	30.7	15.1	29.3	19.7	—	5.0	—	—	4.6	1.0	18.5	11.6
Total cyclics	65.4	38.7	58.3	60.0	—	8.4	—	—	66.8	11.2	31.0	27.4
Total *n*-alkanes	0.6	6.8	1.4	0.1	67.1	30.7	37.1	11.3	7.0	3.8	0.7	12.6
Alkane CPI[b]	0.3	0.1	3.6	0.1	0.2	0.9	0.2	0.6	0.1	1.2	—	0.3
Alkanes >C20/≤C20	2.7	1.7	22.2	0.0	15.6	3.9	25.0	1.2	—	1.0	2.6	53.5
Total *n*-fatty acids	4.4	28.1	17.0	4.0	24.7	29.7	37.3	50.5	3.8	25.8	11.3	54.0
Total unsaturated fatty acids	0.2	5.7	0.9	0.5	1.3	1.6	1.8	4.6	0.4	0.6	3.1	1.8
Fatty acid CPI[b]	9.4	4.8	3.1	15.6	17.1	3.1	8.2	7.0	7.0	10.0	18.4	8.7
Fatty acids >C20/≤C20	1.0	1.0	2.5	1.2	0.5	0.4	2.1	0.6	0.4	0.5	0.3	3.9

[a]Relative to the total chromatographic area. Compounds representing less than 0.1% (dashes) were not taken into account. Sample labels refer to Table 1.

[b]Carbon preference index (molecules C_{2n}/molecules C_{2n+1}).

TABLE 4

Tentative assignment[a] of ^{13}C-NMR signals of the lipid fraction[b] from forests soils and plant leaves

ppm	Structure
14	Terminal-CH_3
18	$C_{5\omega}$ in isoprenoid chains
22	-CH_3 in isopropyl groups
23	C_5 *cis* in isoprenoid chains; C_2 in *n*-alkyl; ω_2 in acyl chains
24	C_3 in *n*-acyl chains acids; branched -CH_3 groups
25	-CH_2- in cyclic structures
26	$C_{1\omega}$ in isoprenoid chains
27	$C_{4\omega}$ in isoprenoid; allylic carbons (*cis*) in unsaturated acyl chains
29	-CH_2- in long alkyl chains
32	C_1 *trans-cis* in isoprenoid chains; C_3 in *n*-alkyl; ω_3 in acyl chains
34	C_2 in *n*-fatty acids; C_1 *cis-cis* in isoprenoid chains; allylic carbons (*trans*)
38	-CH_2-*trans-trans*
47	Quaternary carbons
49	Quaternary carbons in hydroaromatic ring systems
50	Tertiary carbons in cycloalkane structures
51	C-α in phenylpropanoid-type structures
52	C-β in phenylpropanoid-type structures
56	Methoxyl carbons
62	C_α in triacylglycerols, C_γ in β-O-4 lignin units; C_1 in long chain alcohols
64	Etherated secondary carbons, C_α in mono and di-acylglycerols
69	Etherated tertiary carbons, Cβ in triacylglycerols
72	Cβ in mono- and di- acylglycerols
122	Olefinic carbons
123	-C=C- in cycloalkene structures
124	C_3 *cis* in isoprenoid units
127	C_1-C_3 in diterpene-like ring systems, C-β in Ar-CH=CH-CH_2OH
128	Unsubstituted aromatic carbons; olefinic carbons in acyl chains
130	C-α in Ar-CH=CH- or ArH; C_4 in diterpenes; olefinic carbons in acyl chains
135	C_2 *cis* in isoprenoid units
150	C_3 and C_5 in syringyl units
174	Carbonyl carbons

[a]In the case of the branched alkyl chains, the carbons are conventionally referred with the nomenclature of the isoprenoid units.

[b]Extracted with petroleum ether (40–70°C).

chain produce signals at 14 ppm for Cω1, ~23 ppm for Cω2, and 32 ppm for Cω3, which coincides with peaks found in all the lipids under analysis. In addition, when unsaturated fatty acids are present, the allylic carbons should contribute to the 27-ppm signal (*cis*) and the 34-ppm signal (*trans*) (Gunstone, 1994, 1999).

Quantitative data for the different types of alkyl carbons (Table 5) show clearly that transformation in the soil in all cases is accompanied by the increase in concentration of polymethylene structures (31–28 ppm) at the expense of the other alkyl C-types, including the other secondary carbons in the 28 to 24-ppm chemical shift range.

Most defined signals in the region for aromatic/unsaturated carbons are at ca. 124, 127, 128, 130, 135, and 150 ppm. The differences between soil and plant lipids were much more marked in this spectral region: In lipids from soils under pine, there was some increase in complexity in relation to plant lipid, whereas the opposite was observed in juniper soils.

That the two major signals in the aromatic-unsaturated spectral range (135 and 124 ppm; Fig. 2) showed low intensity compared with *e.g.*, the published polyprenol spectra suggests that the structures present in soil lipid do not consist primarily of unsaturated chains. In this region, olefinic carbons in unsaturated fatty acids contribute about 130 ppm to the signal intensity (Polesello et al., 1993; Gunstone, 1994).

In the case of pine samples, a different tendency was observed in the concentration of heterosubstituted structures in the soil samples, which could be interpreted as a relative increase of resins. This is not observed in juniper soils, probably because of the comparatively greater reactivity of the labdanoic skeleton, which agrees with the GC/MS data in Table 3.

The O-alkyl region (46–110 ppm) showed common spectral features in soil and plant lipids. In addition to the 56-ppm methoxyl peak, there was no typical signal pattern attributable to structures of sugars (which would be expected from glycolipid residues) or side-chains of lignin methoxyphenols (present in oligomer residues extracted from soil or plant materials). The most frequent and intense signals appeared at ca. 62 and 64 ppm, which coincided with those characteristic of acylglycerols (Gunstone, 1994); the soils under juniper also showed defined peaks at 69 and 72 ppm. All of these signals showed the same chemical shifts as those of triglycerides as well as mono- and diglycerides. The signals at around 62 and 69 ppm coincided with those of the Cα and Cβ of triacylglycerols, respectively, whereas the shifting of the above signals at ca. 64 and 72 ppm is typical of Cα and Cβ carbons in mono- and di-acyl glycerol esters (Gunstone, 1994). Apart from the possible contribution of acylglycerols, the presence of esters, including high molecular weight waxes and estolides, is well documented in pine and Cupressaceae leaves (Walton, 1990)

TABLE 5

Integration data[a] for the most representative regions of the ^{13}C-NMR spectra of soil and plant lipids

	Plant lipids	Soil lipids			Plant lipids	Soil lipids			Plant lipids	Soil lipids		
	P	P1	P2	P3	O	O1	O2	O3	J	J1	J2	J3
Carbonyl (200–160 ppm)	2.4	0.9	5.8	2.4	0.6	2.7	0.9	4.1	4.3	1.3	2.8	1.6
Total aromatic/alkenoic (160–110 ppm)	18.3	6.5	16.0	10.3	9.1	5.6	3.1	3.3	9.9	4.4	3.7	3.1
O,N-aromatic/alkene (160–140 ppm)	0.8	1.6	4.8	2.0	1.0	1.7	1.6	1.5	1.9	1.2	0.6	0.7
H-aromatic/alkene (140–110 ppm)	17.5	4.9	11.2	8.3	8.1	1.9	1.5	1.8	8.0	3.2	3.1	2.4
O-alkyl (110–46 ppm)	6.7	6.0	14.6	9.9	5.0	1.2	6.0	6.2	12.5	6.8	5.2	7.4
Total alkyl (46–0 ppm)	72.6	86.6	63.6	77.4	85.9	91.0	90.0	86.6	73.3	87.5	88.3	87.9
Quaternary alkyl carbons (46.0–35.0 ppm)	6.7	4.7	5.2	8.2	4.0	6.2	2.6	1.1	6.3	2.5	6.2	3.1
Tertiary alkyl carbons (35.0–30.5 ppm)	9.1	11.7	7.4	7.5	5.8	11.0	6.3	7.1	7.6	5.9	12.8	8.8
Alkyl [polymethylene] (30.5–27.5 ppm)	21.3	42.0	34.2	31.2	34.2	54.7	61.0	61.6	31.8	64.1	41.5	53.3
Secondary alkyl carbons (27.5–24.0 ppm)	14.2	7.5	4.6	7.5	16.8	6.8	6.9	3.3	11.2	3.8	8.4	11.2
Primary alkyl carbons (24–0 ppm)	21.3	20.7	12.2	23.0	25.1	14.3	13.2	13.5	16.4	11.2	19.4	11.5

[a]Percentage of the total spectral area, not considering the contribution by solvent ($CDCl_3$) signals.

and may be invoked to explain the presence of well defined signals in the O-alkyl region.

DISCUSSION

When the differences in ^{13}C-NMR signal intensity between lipids from soils under different vegetation were analyzed using multivariate statistical procedures, the potential of ^{13}C-NMR to reveal differences in terms of the lipid was discovered. Discriminant analyses showed not only the expected differences between plant lipid and soil lipid but also differences regarding the three types of vegetation analyzed. Figure 4 shows the scatterdiagrams obtained using the peak area measurements as descriptors and the vegetation type as the classification factor. It was evident that even the data obtained for the lipid subspectra corresponding to the different types of carbons were useful ($P < 0.05$) in recognizing the original forest on the soil. Inasmuch as the coefficients were calculated from standarized data, after backwards automatic selection of variables, the different coefficients of the discriminant functions indicate the most diagnostic NMR peaks in terms of vegetation.

On the other hand, that NMR spectra suggest a large variety of structures that are not apparent in the standard GC/MS analysis. These could be interpreted as the presence in the lipid samples of a large number of nonvolatile structures that include inherited transformed esters that could have survived the microbial saponification in soil, as well as much more complex material, such as very long-chain individual molecules and/or highly branched polyalkyl backbones including unsaturated and cyclic structures. For the signals in the O-alkyl region, a decreased intensity observed in the O and J series showed a loss of these structures.

In particular, the ^{13}C-NMR spectra resemble those from the material removed from humic acids with organic solvents by supercritical gas extraction (Schnitzer and Preston, 1987), which suggests complex aliphatic material of a nature similar to that described in the present study as well as those obtained by Amblès et al. (1991) for the polar soil lipid fraction. These authors suggest that from a molecular viewpoint, the most complex portion of the lipid fraction shows features in common with the protokerogen fraction of sedimentary organic matter formed by the selective preservation of resistant biomacromolecules (Hatcher et al., 1980; Tegelaar et al., 1989; de Leeuw et al., 1991; Amblès et al., 1993).

In conclusion, soil lipids represent an interesting soil fraction. These include low molecular weight biomarker products in addition to

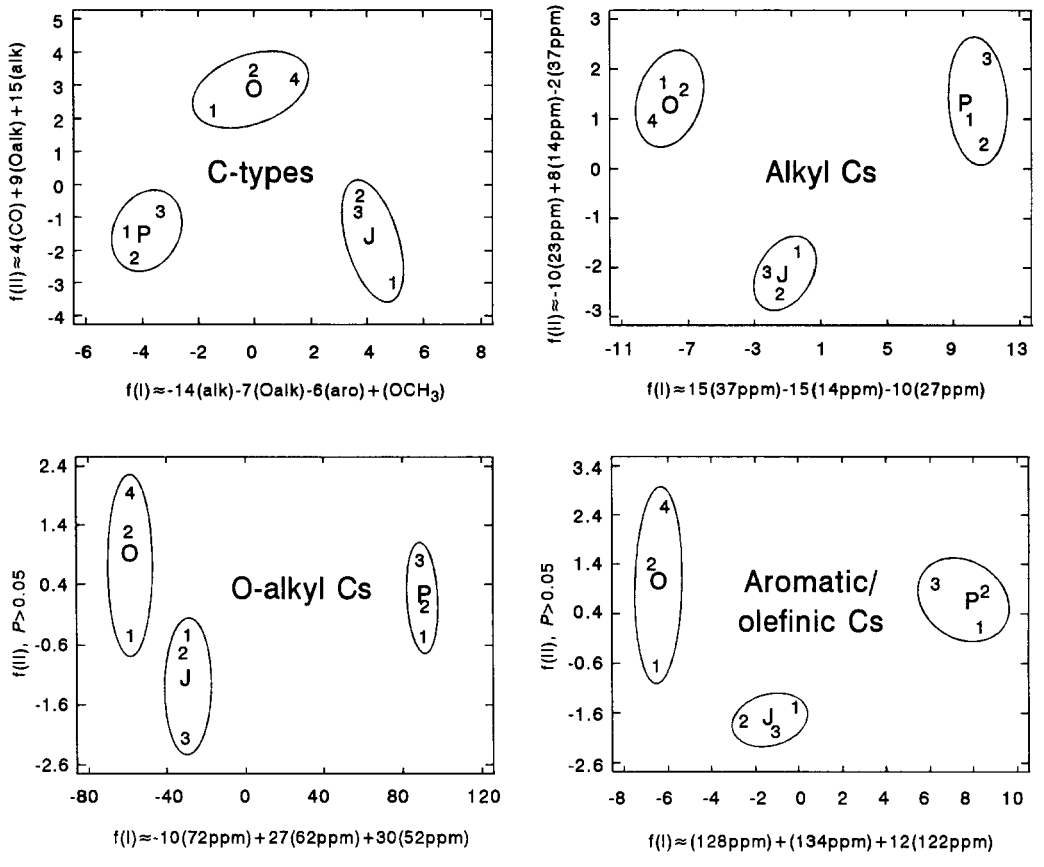

Fig. 4. In the space defined by the two functions obtained by factorial discriminant analysis, representation of the points corresponding to lipid samples using as original descriptors the peak area of the main ^{13}C-NMR spectra of soil lipids from three groups of forest soils (P = pine forest, O = oak forest, J = juniper forest. Labels located at the cluster centroids).

nonvolatile products that can be analyzed by ^{13}C-NMR and that show diagnostic features in terms of vegetation.

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