

Effects of Fire on Humic and Lipid Fractions in a Dystric Xerochrept in Spain

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ABSTRACT

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Fire-induced changes in humic and lipid soil fractions were assessed by two approaches. Comparisons were made of the nature and amounts of the two fractions in samples of a Dystric Xerochrept under two pine stands, one that had been burned two years earlier and one that had not. Further comparisons were made of humic and lipid fractions in a soil sample before and after heating in the laboratory. Separation and identification of the compounds in these soil fractions were carried out by preparative liquid chromatography followed by GC-MS in the case of lipids; the humic acids were previously degraded by successive treatments with sodium persulphate and potassium permanganate.

The greatest differences were in the distribution patterns of soil lipids (alkanes, fatty acids): accumulation of the homologues of lower molecular weight ($< C_{20}$) was dominant in the post-fire samples. For soil resin acids, these samples had higher values for dehydroabietic and secodehydroabietic acids and lower ones for pimaric acid.

The degradative studies on the humic acids showed that the relative yields of aromatic products were comparatively higher in the samples after burning. Alkanoic diacids were found to be the least resistant to heating, whereas greater amounts of alkanes in the less condensed polymer structures of the humic acids were observed in the samples after burning. The relative amounts and distribution patterns of the fatty acids released showed no great differences in the humic acids.

INTRODUCTION

The biomass and the organic horizons of the soil may be burned periodically because of the pyrophytic character of several plant communities and the dry, hot summers in Mediterranean ecosystems. Under these conditions, the influence of forest fires on the evolution of soil humus may be greater than the biological, geological and climatic factors, producing lasting changes in the physical and chemical properties of the soil (Viro, 1974). Changes in the total amounts of organic carbon, nitrogen, litter, humic fractions and water-repel-

lent substances have been described in burned areas (De Bano et al., 1970; Savage, 1974; Giovannini et al., 1983; Almendros et al., 1984a; Giovannini and Lucchesi, 1984; Vega et al., 1985), but the study of the effects of forest fires on the chemical nature of those soil fractions has not received great attention. Intense or repeated burning may lead to the differentiation of 'pyromorphic' types of humus, the characteristics of which may, in several cases, be compared with those attributed to active processes of biodegradation and humification (decreases in the C/N ratio, increases in the humin and humic acid proportions, etc.) (Almendros et al., 1984b). The interpretation of the characteristics of humus in soils under burned forest, in which organic matter is mineralized or transformed in part and at the same time replaced in part by the fall of incompletely burned plant tissues, may be simplified by performing parallel laboratory-controlled experiments, where the effects of burning on humus ('fire intrinsic effects') could be studied independently of the incorporation of new types of more or less transformed organic matter.

The present article describes the effects of laboratory burnings and forest fire on humic acids and lipid compounds in forest brown soil (Dystric Xerochrept) under *Pinus pinea*.

MATERIALS

At two different sites, 10 soil samples were collected to represent the O + A1 horizons of a Dystric Xerochrept under *Pinus pinea* in Madrid, Spain. The first site was in a part of the forest that had been subject to a fire of medium intensity two years earlier. The second site was in a part of the forest that had not been burned. The burned and control sites were separated by a forest road, which was approximately 3 m wide. Samples were prepared after mixing 5 soil samples (to a depth of 15 cm), randomly collected in every site (approximately 30 m²). The sample representative of the original forest soil is identified by the letter C and also called the "control sample", whereas the sample prepared from the post-fire site is called B. Sampling took place in the spring; after eliminating the litter layer, approximately 5 kg of soil was taken with a spade from each sampling point.

Subsamples of sample C were heated to a pair of temperatures in the laboratory, following an experimental procedure previously described (Almendros et al., 1984b). One was heated to 160°C and is identified by the combination C-I, whereas the other was heated to 210°C and is identified by the combination C-II. The conditions of these samples were considered to be comparable to those reported for wildfires and controlled burnings with regard to fire intensity and depth (De Bano et al., 1977; Vega, 1985). Several analytical characteristics of the soil samples are given in Table I.

TABLE I

Analytical characteristics of control and heated soil samples

Soil sample	Color (Munsell)	pH	T	S	% C	C/N	Free organic matter ^{*1}	Humic acid ^{*1}	Fulvic acid ^{*1}
C (Control forest)	5YR 6/3	5.73	24.6	14.3	3.02	12.3	1.94	0.20	0.22
B (Burned forest)	10YR 5/1	6.57	40.2	29.2	3.98	10.6	2.34	0.55	0.23
C-I (laboratory heated to 160°C)	10YR 4/3	5.80	23.1	12.5	2.70	13.2	1.51	0.19	0.22
C-II (laboratory heated to 210°C)	10YR 4/3	6.00	25.3	10.2	2.53	13.4	1.20	0.18	0.18

T=total exchange capacity (pH=8), meq. per 100 g; S=total exchangeable basis, meq. per 100 g.

^{*1}g C per 100 g soil.

METHODS

Organic fragments were removed by flotation, and lipid extraction was carried out in a Soxhlet with light petroleum for a week. Humic acids (HA's) were then extracted with 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ and 0.1 M NaOH under nitrogen. HA was recovered after precipitation with HCl, purified by repeated centrifugation at 29,000 g and treatments with 1 M HCl-HF (1:1) and by dialysis.

HA's were methylated (Schnitzer, 1974) then degraded with potassium persulphate (Martín et al., 1981); the non-degraded residue was subsequently oxidized with potassium permanganate until total degradation (Matsuda and Schnitzer, 1972). Because of the mild character of the former reagent as compared to the latter, the resulting oxidation products were considered as produced by the loosely associated and more strongly linked structural components of the humic heteropolymers, respectively.

In all the cases, degradation products were extracted with ethyl acetate, dehydrated with anhydrous Na_2SO_4 , dried under N_2 , methylated with diazomethane, and purified by column chromatography (silica gel 0.05–0.2 mm, 7734 Merck) using methylene chloride–hexane 5:1 as eluent, to remove the non-volatile, oligomeric coloured fractions before the GC-MS analyses. In the case of soil lipid compounds, neutral and polar fractions were separated in the silica gel column, using successively hexane and hexane–methylene chloride 1:1. Quantitative yield with respect to the initial weight of sample was not calculated, following the criteria of Maximov et al. (1977), but relative percentages with respect to the total volatile products were used for comparisons between chromatograms.

Separation of the degradation compounds and of the lipid fractions were made with a Hewlett-Packard 5992 B gas-chromatographic mass-spectrometric computer system with a 25-m cross-linked OV-101 capillary column. Oven temperature was programmed to increase from 100 to 270°C at a heating rate of 6°C min⁻¹. Helium flow was 1 ml min⁻¹, and ionizing voltage in the MS was 70 eV. The identification of the chromatographic peaks was carried out

after previous matching of their mass spectral patterns with those in a database for methyl esters of soil compounds created in a personal computer from the collection of data found in the bibliography or obtained after injection of standards. In the case of terpenoid compounds, definitive assignment was only given after significant correlation (> 0.9) of their spectral data and coincidence with the elution sequence reported in Zinkel et al. (1971), Ekman (1979), and Simoneit and Mazurek (1982).

RESULTS AND DISCUSSION

Lipid fractions

The accumulation of these hydrophobic substances was noticeable in the soil sample from the burned forest, where the lipid fraction was more than 6 times greater in weight than in the control sample (Fig. 1). This difference was not observed under laboratory conditions. It is attributed to translocation into the soil of organic substances released from burning litter or biomass (De Bano et al., 1970, 1976). The formation of non-wettable soils as a consequence of forest fires is partially due to the occurrence of similar phenomena (Savage, 1974; Giovannini and Luchessi, 1984).

Major constituents of the hydrocarbon fraction (Fig. 2) were C_{14} to C_{33} linear alkanes, some branched alkanes, and a terpene of $MW = 256$ (comp. 46 in Table II). The mass spectrum of this compound presented a base peak of m/e 159 and other characteristic peaks at m/e 241, 185, 117 and 129, and was identified as dehydroabietin. The same compound was also reported in soils by Swan (1965) and by Simoneit and Mazurek (1982) who considered it as a

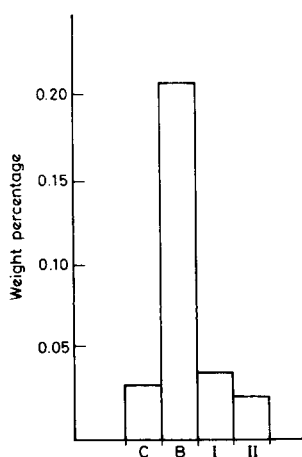


Fig. 1. Total lipid content of control sample (C), sample from post-fire site (B) and control soil samples after heating in laboratory (I, II).

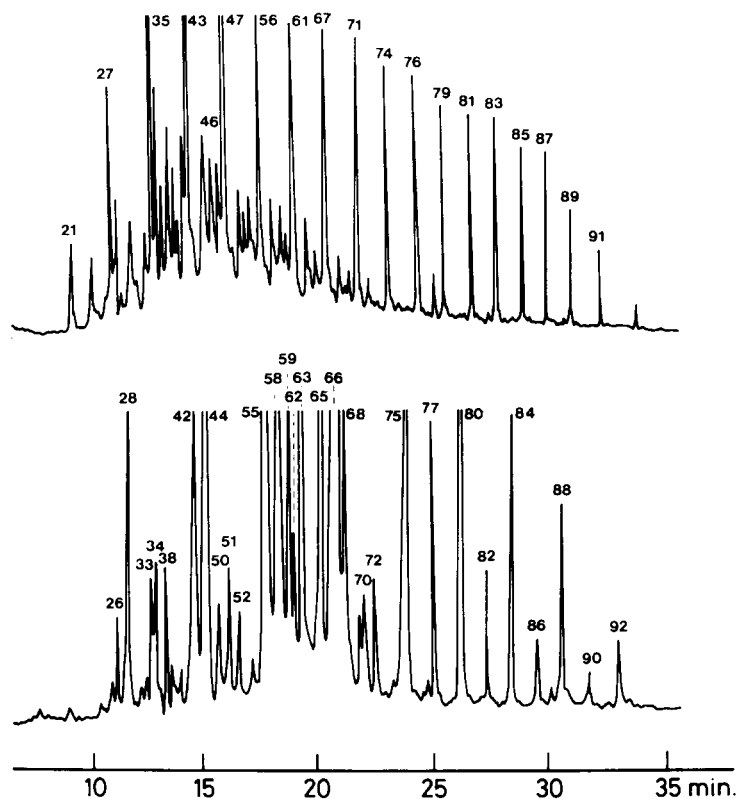


Fig. 2. Gas chromatographic separation of soil lipid fractions. Above: neutral fraction of heated sample C-II (unlabelled peaks corresponded to different branched alkanes). Below: fraction eluted by methylene chloride (mainly fatty and resin acids) of the control sample.

diagenetic product of resin acids. This hydrocarbon (in relation to alkanes) was found to be very resistant to burning in the laboratory. A similar resistance was observed for the branched alkanes, the relative proportions of which did not differ significantly in the studied conditions.

The differences in the distribution patterns of *n*-alkanes in the samples were important (Fig. 3). The alkane series of the control soil sample (C) showed a bimodal distribution with a maximum in the C_{29} and a predominance of the odd-C numbered chains (even/odd ratio=0.6), which suggests a significant contribution of cuticular waxes of higher plants to the soil lipid fraction (Simoneit and Mazurek, 1982). In the sample from the post-fire site (B), there were higher amounts of the alkanes with lower molecular weight ($< C_{25}$) and no predominance of the odd-C numbered chains; the maximum was found in the C_{18} – C_{20} region (total even/odd ratio=0.8). Similar differences were found in comparison of the alkane series of the control sample with those of the samples C-I and C-II. In the first case, the lengths of the chains showed small

TABLE II

Organic compounds contributing to soil lipid fractions and to degradation products of humic substances

No. Compound* ¹	No. Compound* ¹
(1) Dimethyl glutarate	(31) Methoxy benzene dicarboxylic acid dimethyl ester (II)
(2) Methoxy benzenecarboxylic acid methyl ester (I)* ²	(32) 1,2,3 Benzene tricarboxylic acid trimethyl ester
(3) Methyl 3 phenyl propionate	(33) 15-C branched fatty acid methyl ester (I)
(4) Methoxy benzenecarboxylic acid methyl ester (II)	(34) 15-C branched fatty acid methyl ester (II)
(5) Dimethyl pimelate	(35) <i>n</i> -Octadecane
(6) Methoxy benzenecarboxylic acid methyl ester (III)	(36) 1,2,4 Benzene tricarboxylic acid trimethyl ester
(7) Propane tricarboxylic acid trimethyl ester	(37) 12-C Branched dicarboxylic aliphatic acid dimethyl ester
(8) <i>n</i> -Tetradecane	(38) Methyl pentadecanoate
(9) 1,2 Benzene dicarboxylic acid dimethyl ester	(39) Branched alkane
(10) Dimethoxybenzaldehyde	(40) <i>o</i> -Terphenyl
(11) 8-C Branched dicarboxylic aliphatic acid dimethyl ester	(41) Benzene tricarboxylic acid trimethyl ester
(12) Dimethyl suberate	(42) Methyl hexadecenoate
(13) 1,3 Benzene dicarboxylic acid dimethyl ester	(43) <i>n</i> -Nonadecane
(14) 1,4 Benzene dicarboxylic acid dimethyl ester	(44) Methyl hexadecanoate
(15) <i>n</i> -Pentadecane	(45) Methoxy benzene tricarboxylic acid trimethyl ester (I)
(16) Paratoluensulphonic acid methyl ester	(46) 18-Norabieta-8,11,13-triene
(17) 9-C Branched dicarboxylic aliphatic acid dimethyl ester	(47) <i>n</i> -Eicosane
(18) Methyl dodecanoate	(48) Methoxy benzene tricarboxylic acid trimethyl ester (II)
(19) Dimethyl azelate	(49) 17-C unsaturated fatty acid methyl ester
(20) Dimethoxy benzenecarboxylic acid methyl ester	(50) 17-C branched fatty acid methyl ester (I)
(21) <i>n</i> -Hexadecane	(51) 17-C branched fatty acid methyl ester (II)
(22) 10-C Branched dicarboxylic aliphatic acid dimethyl ester	(52) Methyl heptadecanoate
(23) Dimethyl decanedioate	(53) Dimethyl tetradecanedioate
(24) Methoxy benzene dicarboxylic acid dimethyl ester I)	(54) 1,2,3,4 Benzene tetracarboxylic acid tetramethyl ester
(25) Methyl <i>p</i> -methoxy cinnamate	(55) Methyl octadecenoate
(26) 14-C unsaturated fatty acid methyl ester	(56) <i>n</i> -Heneicosane
(27) <i>n</i> -Heptadecane	(57) 1,2,4,5 Benzene tetracarboxylic acid tetramethyl ester
(28) Methyl tetradecanoate	(58) Methyl octadecanoate
(29) 11-C Branched dicarboxylic aliphatic acid dimethyl ester	(59) Methyl 2S-(2' (m-isopropylphenyl)ethyl)-1R,3S-dimethyl cyclohexanecarboxylate
(30) Dimethyl undecanedioate	

TABLE II (continued)

No. Compound* ¹	No. Compound* ¹
(60) 1,2,3,5 Benzene tetracarboxylic acid tetramethyl ester	(77) Methyl tricosanoate
(61) <i>n</i> -Docosane	(78) Branched alkane
(62) Methyl nonadecanoate	(79) <i>n</i> -Heptacosane
(63) Methyl 8(14),15-pimaradien-18-oate	(80) Methyl tetracosanoate
(64) Dimethyl hexadecanedioate	(81) <i>n</i> -Octacosane
(65) Methyl 8,12-abietadien-18-oate	(82) Methyl pentacosanoate
(66) Methyl 8,11,13-abietatrien-18-oate	(83) <i>n</i> -Nonacosane
(67) <i>n</i> -Tricosane	(84) Methyl hexacosanoate
(68) Methyl eicosanoate	(85) <i>n</i> -Triacontane
(69) Dioctyl adipate	(86) Methyl heptacosanoate
(70) Methyl 7-hydroxydehydroabietate	(87) <i>n</i> -Hentriacontane
(71) <i>n</i> -Tetracosane	(88) Methyl octacosanoate
(72) Methyl heneicosanoate	(89) <i>n</i> -Dotriacontane
(73) Dimethyl octadecanedioate	(90) Methyl nonacosanoate
(74) <i>n</i> -Pentacosane	(91) <i>n</i> -Tritriacontane
(75) Methyl docosanoate	(92) Methyl triacosanoate
(76) <i>n</i> -Hexacosane	(93) <i>n</i> -Tetratriacontane

*¹Methyl esters.*²Roman numbers indicate different isomers.

alterations, but the relative proportion in the even-C numbered alkanes increased (even/odd ratio=0.9). At higher temperatures (sample C-II), the chains with a high molecular weight decreased; the maximum shifted to C₂₉ as in sample B and the odd-C number preference totally disappeared (even/odd ratio=1.1). Gas-chromatograms of this post-fire sample (Fig. 2) are also comparable to those of a non-biogenic alkane series, and the main difference with respect to sample B was that in the latter the proportions of the longer chain, odd-C numbered alkanes remain high as a possible effect of the translocation and fixation in soil of substances released during the burning of litter (De Bano et al., 1970, 1976, 1977).

Differences observed in the more polar lipid fraction were also important (Figs. 2, 3 and 4). This fraction consisted mainly of fatty acids and resin acids. In all the cases studied, the fatty acids ranged from C₁₄ to C₃₀; in the case of the control sample (Figs. 2 and 3) the maximum was found in the C₁₆ acid, but the relative proportion of the longer-chain acids (> C₂₀) was important, which also confirmed a considerable contribution from waxes of higher plants to soil lipids. In the case of sample B, the total proportion of high MW acids was similar to that of the C sample, but higher in individual cases (C₂₂ and C₂₄). When the subsamples (C-I, C-II) were heated in the laboratory, chains of lower molecular weight (< C₂₀) were increased in amount. In the subsample C-II

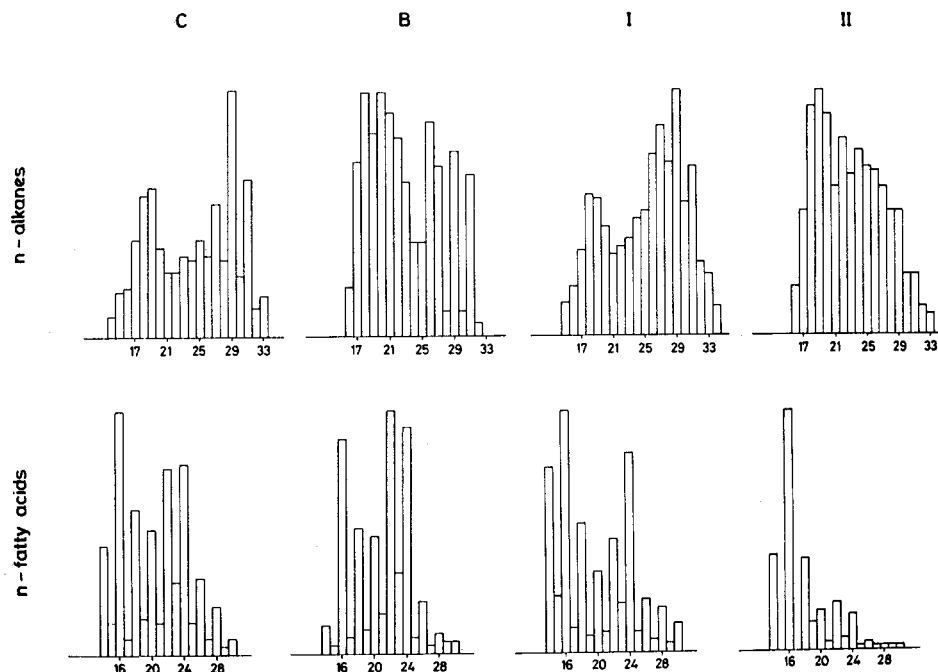


Fig. 3. Distribution diagrams (relative abundance vs. C number) for *n*-alkanes (above) and *n*-fatty acids (below) extracted from soil samples (C, control sample; B, sample from post-fire site; I, II, laboratory-heated soil samples).

heated to the higher temperature, the dominant peaks were for C_{16} , C_{14} and C_{18} acids.

Different conifer resin compounds (Zinkel et al., 1971) or their derivatives were identified in this polar lipid fraction, the most abundant being the dehydroabietic acid (comp. No. 66). Pimaric acid (No. 63) an abietadienoic acid (No. 65) and a hydroxydehydroabietic acid (No. 70; Ekman, 1979) were also detected. An unusual resin acid (comp. No. 59) was also found in all the samples. This compound ($MW = 316$) presented an MS with a base peak at m/e 146, and major peaks at m/e 133, 101 and 187. Due to the absence of peaks at m/e 181 and 121 (absence of C_9-C_{10} bond *beta* to a double bond system), and the comparatively low retention time of this compound, it was considered as a secodehydroabietic acid (Takeda et al., 1968; Zinkel et al., 1969). Another compound identified in this fraction was terphenyl (No. 40), which appeared only in sample B.

Relative proportions of the different terpenoids are shown in Fig. 4. Although these quantitative data may be affected by the transformations and isomerizations during the extraction procedures and gas chromatographic processing (Zinkel et al., 1971; Chang et al., 1971), they may be considered as

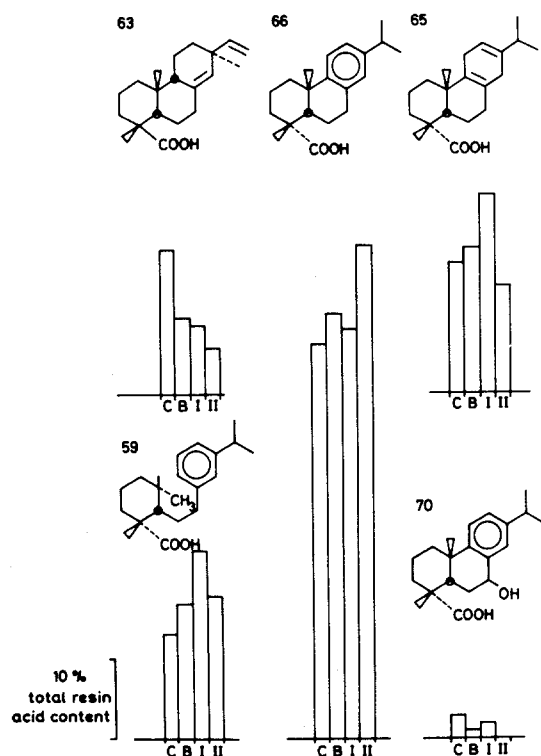


Fig. 4. Relative proportion of soil resin acids (C, control sample; B, sample from post-fire site; I, laboratory-heated soil sample; II, laboratory-heated soil sample). Compound numbers refers to Table II.

comparable in the samples described. Pimaric acid showed the lowest resistance to burning, and its relative proportion decreased in the laboratory heating. In the case of hydroxydehydroabiatic acid, the proportion was small in all cases. The amount of dehydroabiatic acid, the most abundant resin acid, increased relatively under laboratory heating, perhaps as a consequence of transformations of the other resin acids into dehydroabiatic acid, as described for the natural diagenetic pathways of this kind of compounds (Simoneit and Mazurek, 1982). On the other hand, the important amounts of secodehydroabiatic acid in samples after burning might be partially due to the thermal ring opening of other resin acids, as described by Takeda et al. (1968), but in sample C-II the relative increase was reduced, as in the case of the other resin acids except dehydroabiatic acid.

Degradation products of humic acids

Persulphate treatment chiefly removed aliphatic molecules: fatty acids, alkanes and alkanolic diacids (Figs. 5 and 6). More than 25% of aromatic prod-

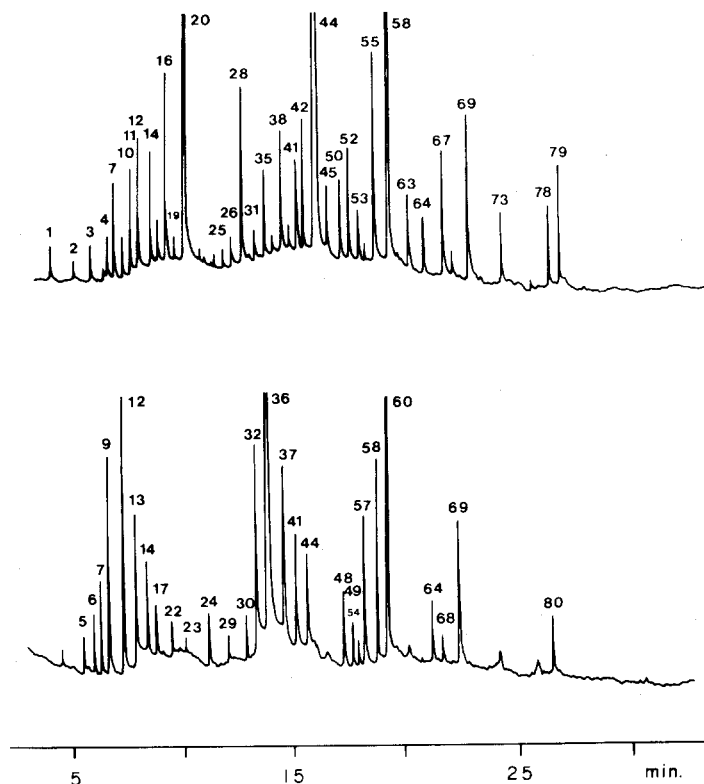


Fig. 5. Gas chromatographic separation of degradation products of humic acid from the burned site (sample B). Above: products obtained after acid persulphate degradation. Below: products obtained after alkaline permanganate oxidation of the persulphate residue.

ucts (benzenecarboxylic and phenolic acids) were also obtained: a dimethoxybenzenecarboxylic acid (comp. No. 20) was the most abundant product released by persulphate oxidation in the control sample (C). Other important products were propanetricarboxylic acid (No. 7), not found in sample C-II, p-methoxy cinnamic acid (No. 25), phenyl propionate (No. 3), compounds probably released during the lignin degradation processes. Pimaric acid was also detected in the persulphate digests, being probably entrapped in the humic structures, or perhaps linked by its carboxyl group (No. 63), but the amounts of this compound tend to be comparatively small in samples B, C-I and C-II.

Distribution patterns of fatty acids associated with HA's showed no important differences between the control sample and the samples after heating. Cumulative representation (Fig. 6) showed that the total amount of alkanoidiacids decreased, whereas the fatty acid content was very constant even after the more severe treatment (C-II). The proportion of *n*-alkanes in forms re-

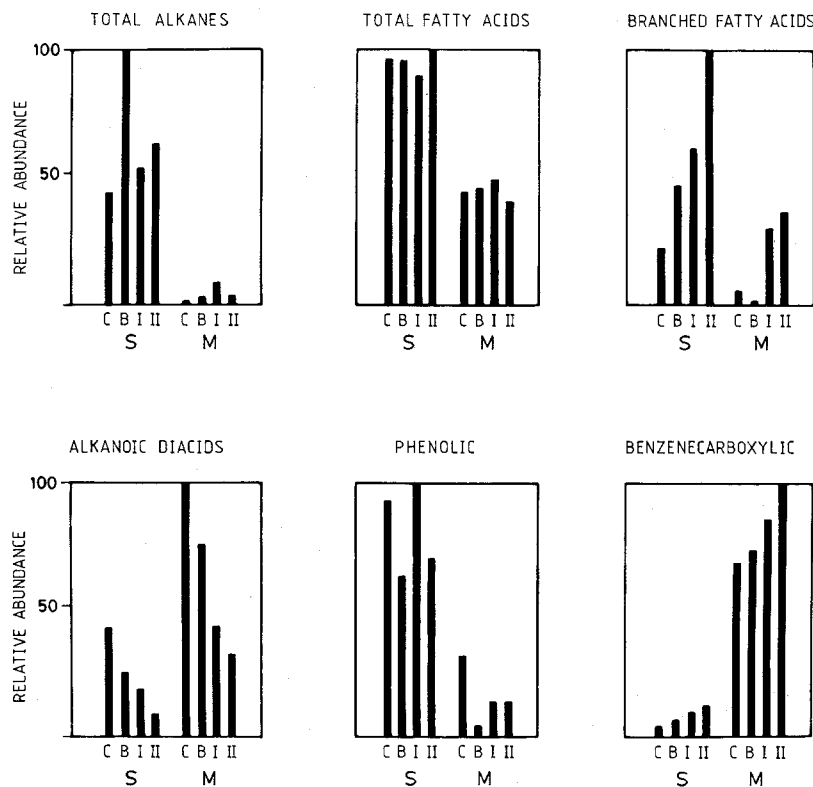


Fig. 6. Proportional content of the major groups of organic compounds obtained after successive degradation of humic acids with $K_2S_2O_8$ (S) and $KMnO_4$ (M) (C, control sample; B, sample from post-fire site; I, II, laboratory-heated soil samples).

leased by persulphate tends to be comparatively higher in the samples after heating. This fact partially offset the relative decrease of the aliphatic character of the whole polymer due to heating, mainly due to selective destruction of the structures producing alkanolic diacids. Branched or saturated fatty chains were more resistant in the conditions studied, and the benzenecarboxylic/phenolic ratio tended to be higher in the sample from post-fire site and the samples heated in the laboratory.

After permanganate degradation, the main peaks were different isomers of benzenepolycarboxylic acids, which represented more than half of the obtained products. In all the samples, the major compound was the 1,2,4 benzenetricarboxylic acid. Propanetricarboxylic acid was abundant in the control sample (8%) but lower in the samples B, C-I and C-II. As in the case of the persulphate degradations, the series of fatty acids bonded to HA's presented a pattern relatively constant in the control and the heated samples (mainly the saturated and branched chains); the reduction in the alkanolic diacid fraction and the

noticeable increase in the benzenecarboxylic/phenolic ratio was also confirmed in these more stable compounds of the humic polymer.

CONCLUSIONS

Analytical differences observed after comparisons of the control soil sample with those affected by burning under natural or laboratory conditions were higher in the case of lipid fractions than in HA's. In the case of soil lipids, a tendency towards a selective increase in amounts of the compounds of a shorter chain length was a consequence of the intrinsic effects of fire, whereas lipid compounds associated to HA's were found to be comparatively more stable when heated.

The relative proportions of the diterpene resin acids differed significantly in the samples studied. This seems a probable consequence of thermal isomerizations and transformations such as ring opening of different types but also by selective volatilization of different compounds. Because thermal increases or decreases of the corresponding terpenoids were not always correlated with their boiling points, it probably had a higher relevance to the first-mentioned phenomenon. The increase observed in the dehydroabietic acid proportion is also a trend shown by the natural diagenetic pathways of these biogenic markers (Weete, 1976; Simoneit and Mazurek, 1982).

For the HA's, the relative amounts of aromatic compounds with a high degree of oxidation produced upon alkaline permanganate degradation were found to be greater in the samples after heating than in the control sample. This is attributed to selective thermal degradation of aliphatic structures (mainly those producing alkanolic diacids). In addition, an accumulation of alkanes in loosely associated forms was observed in the HA's from the heated soil samples, which might be partially due to thermal translocations of litter substances occurring at fire temperatures (De Bano et al., 1976).

The higher values for the yield of aromatic products and for the benzenecarboxylic/phenolic ratio, which have been considered a characteristic of the more biologically transformed types of humus (Schnitzer, 1977), were also found to characterize the heated samples, whether heating was due to a forest fire or to laboratory treatment.

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