

## Comparative Analysis of the Alkyl Breakdown Products from Soil Humic Acids by Thermal and Wet Chemical Degradation Methods

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Total amounts and distribution patterns of alkyl degradation compounds were analyzed in soil humic acids isolated from a variety of continental Mediterranean ecosystems in Central Spain. Soil physical and chemical characteristics, humic acid composition and biomarker assemblages were studied in 16 representative, undisturbed or degraded forest ecosystems including broad-leaved autochthonous forests, bush formations, cultures, pastures and pine forests. The humic acid fraction of these soils was previously characterized by routine spectroscopic methods (UV-visible, FTIR, <sup>13</sup>C and <sup>15</sup>N CPMAS NMR). In the present study qualitative and quantitative features of the alkyl domain of humic acids are examined by comparing the structural information provided by independent destructive techniques, with special emphasis on the mild alkaline degradation (sodium perborate) and thermal degradation by Curie-point pyrolysis.

The structural features of the humic acid matrix (its variable degree of condensation or internal cross-linking) in relation to its potential for molecular encapsulation mainly of compounds lacking reactive functional groups is discussed in the set of soils described in this study: alkane and fatty acid homologue series are compared after direct extraction from soil (hexane extraction), perborate degradation and Curie-point pyrolysis.

Analytical pyrolysis of humic acids yielded a conspicuous C<sub>9</sub>–C<sub>25</sub> series of alkanes, in most cases with the maximum *ca.* C<sub>21</sub>. Excepting some cases in which a clear even-to odd C-number preference is observed (in particular, this situation was found in well-preserved oak forests) in the other alkane series the carbon preference index and their relative chain length indicated that the major constituents have not a recent biogenic origin (*i.e.*, epicuticular waxes of higher plants) but have been probably subjected to intense microbial and abiotic reworking leading to short-chain homologues.

Wet chemical degradation with NaBO<sub>3</sub> shows a different picture of the alkane series, with most samples releasing no substantial amounts of hydrocarbons. Compared to the pyrolytic alkanes, the perborate-released alkanes often show a shifting of the maxima towards the lowest molecular weight (> C<sub>20</sub> alkanes). This suggests that pyrolysis is superior to chemolysis in the case of alkanes because the rapid thermoevaporation probably prevents chain breakdowns and releases entrapped molecules.

The opposed situation occurs with the fatty acids, where pyrolysis yielded narrow series (mainly C<sub>12</sub>–C<sub>18</sub>) with maximum at the C<sub>16</sub> acid. The wet chemical degradation yielded a conspicuous C<sub>12</sub>–C<sub>28</sub> fatty acid series with maximum at C<sub>16</sub> or C<sub>18</sub>, in addition to a series of  $\alpha,\omega$ -alkanedioic acids (up to 40% of the total degradation compounds) including relatively long-chain molecules (> C<sub>9</sub> and frequently > C<sub>12</sub> acids) with characteristic bimodal distribution suggesting specific breakdowns at the level of C<sub>9</sub> and C<sub>4</sub> in-chain substitutions of the most frequent biogenic polyunsaturated fatty acids and their hydroxy-derivatives such are the precursors of cutans, suberans or other natural polyesters. Diagnostic *iso*- and *anteiso*-fatty acids (mainly C<sub>15</sub>) are detected by both chemical and thermal degradation methods.

The alkene series released by pyrolysis (C<sub>6</sub>–C<sub>22</sub>) frequently show a bimodal pattern (up to 5% of the total volatile products, approximately the same proportion as alkanes) and showed no high correlation with other pyrolysis compounds or humic acid parameters independently analysed, what is interpreted as having an origin from heterogeneous structures and/or through independent pathways.

It is assumed that any degradation method produces a more or less intense alteration of the labile aliphatic molecules, thus yielding a biased information about the original alkyl constituents in the humic acid. In the case of pyrolysis, it is probable that a series of hydrocarbons could be readily removed by thermoevaporation and, at least for these alkyl species, the pyrolytic approach would be superior in providing unbiased information.

# COMPARATIVE ANALYSIS OF THE ALKYL BREAKDOWN PRODUCTS FROM SOIL HUMIC ACIDS BY THERMAL AND WET CHEMICAL DEGRADATION METHODS



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Total amounts and distribution patterns of alkyl degradation compounds were analyzed in soil humic acids isolated from a variety of continental Mediterranean ecosystems in Central Spain.

The structural information provided by mild alkaline degradation (sodium perborate) and thermal degradation by Curie-point pyrolysis was compared.

Our results suggest that pyrolysis is a more accurate technique than chemolysis for the analysis of alkanes. The rapid thermoevaporation should prevent chain breakdowns, releasing entrapped molecules. Conversely, perborate degradation is probably giving more information on tightly bonded alkyl structures.

## FOREWORDS

Total amount and distribution patterns of alkyl degradation compounds were analyzed in soil humic acids isolated from a variety of continental Mediterranean ecosystems in Central Spain.



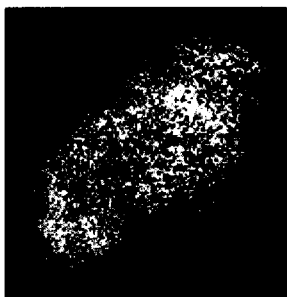
Soil physical and chemical characteristics, humic acid composition and biomarker assemblages were studied in 16 representative, undisturbed or degraded forest ecosystems including broad-leaved autochthonous forests, bush formations, cultures, pastures and pine forests.



The humic acid fraction of these soils was previously characterized by routine spectroscopic methods (UV-visible, FTIR, <sup>13</sup>C and <sup>15</sup>N CPMAS NMR).

In the present study, qualitative and quantitative features of the alkyl domain of humic acids are examined by comparing the structural information provided by independent destructive techniques, with special emphasis on the mild alkaline degradation (sodium perborate) and thermal degradation by Curie-point pyrolysis.

## MODELLING THE SPECIATION OF ALIPHATIC COMPOUNDS IN HUMIC MACROMOLECULES



The structural features of the humic acid matrix (its variable degree of condensation or internal cross-linking) in relation to its potential for molecular encapsulation, mainly of compounds lacking reactive functional groups is considered to explain the behaviour of the alkyl compounds against different degradation methods.

Alkane and fatty acid homologue series are compared after:

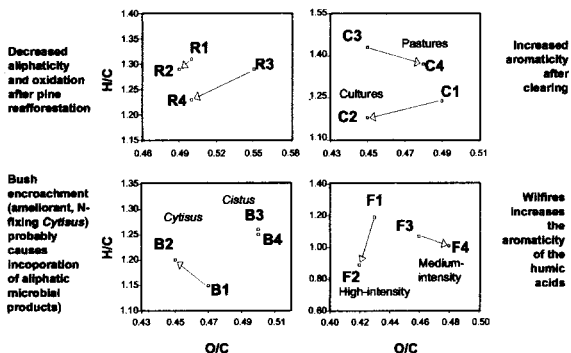
- I) Direct extraction from soil (hexane extraction),
- II) Perborate degradation and
- III) Curie-point pyrolysis

## CHARACTERISTICS OF THE SAMPLING SITES

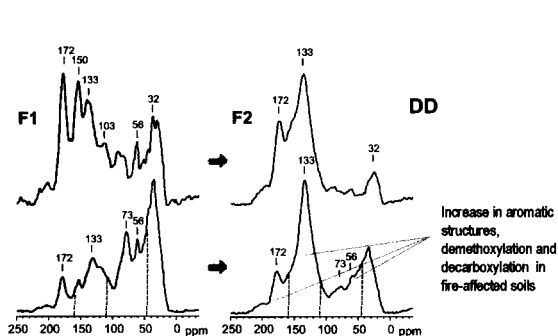
Sample	Vegetation	Site	High (mes)	Geological substrate	Soil type (FAO)
R1	Oak forest ( <i>Quercus ilex</i> )	Galapagar	880	Granite	Ortheutric Cambisol
R2	Pine forest ( <i>Pinus pinna</i> )	Galapagar	855	Granite	Ortheutric Cambisol
R3	Oak forest ( <i>Quercus pyrenaica</i> )	Zarzalejo	1150	Granite	Mollic Cambisol
R4	Pine forest ( <i>Pinus pinaster</i> )	Zarzalejo	1240	Granite	Ortheutric Cambisol
C1	Oak forest ( <i>Quercus ilex</i> )	Nuevo Baztán	870	Limestone	Calcic Cambisol
C2	Cereal cultures	Nuevo Baztán	870	Limestone	Rendzic Leptosol
C3	Ash forest ( <i>Fraxinus angustifolia</i> )	El Berrueco	950	Granite	Epligeyc Cambisol
C4	Pastured site	El Berrueco	956	Granite	Ortheutric cambisol
B1	Chestnut forest ( <i>Castanea sativa</i> )	Rozas Puerto Real	840	Granite	Mollic Cambisol
B2	Brushwood ( <i>Cytisus scoparius</i> )	Rozas Puerto Real	825	Granite	Orthoystic Cambisol
B3	Oak forest ( <i>Quercus ilex</i> )	El Escorial	1015	Granite	Orthoystic Cambisol
B4	Brushwood ( <i>Cistus ladanifer</i> )	El Escorial	990	Granite	Ortheutric Cambisol
F1	Pine forest ( <i>Pinus halepensis</i> )	S. Martín de la Vega	630	Limestone	Calcic Cambisol
F2	Burned site ( <i>Pinus halepensis</i> )	S. Martín de la Vega	824	Limestone	Calcic Cambisol
F3	Pine forest ( <i>Pinus sylvestris</i> )	Somosierra	1580	Gneiss	Umbrithumic Umbrisol
F4	Burned site ( <i>Pinus sylvestris</i> )	Somosierra	1615	Gneiss	Eplidystic Cambisol

Indicates undisturbed soil

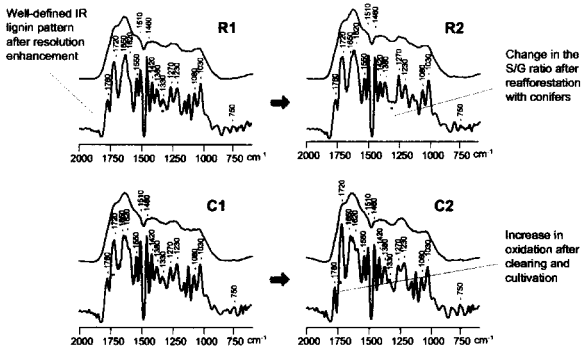
## Van Krevelen plots (H/C vs. O/C atomic ratios) of humic acids



## <sup>13</sup>C NMR spectra (+ dipolar-dephased spectra) from humic acids (fire-affected samples F1 and F2)

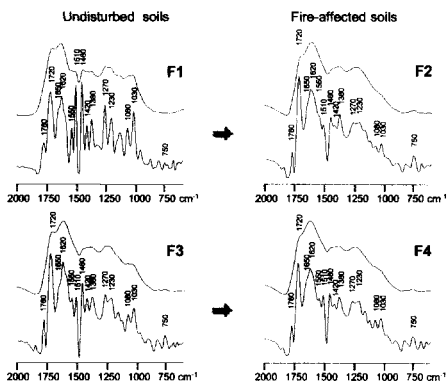


### Resolution-enhanced\* infrared spectra of humic acids (R1, R2, C1 y C2)



\* (subtraction of the original spectrum from a positive multiple of the second derivative)

### RE-IR spectra of humic acids:

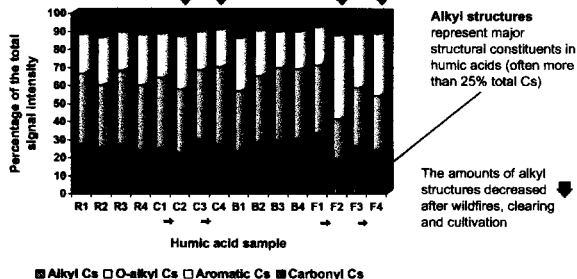


The 2nd derivative visible spectra of HAs (and fungal melanins) suggest the extent of the humic structural moiety with dihydroxyperylenequinone-containing pigments synthesized by soil microorganisms

→ Either reafforestation with gymnosperms or bush encroachment lead to HAs with lower amounts of fungal pigments

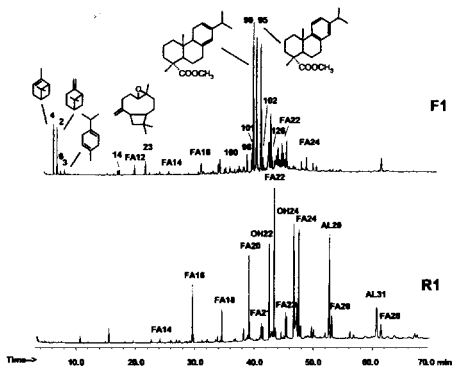
→ Cleared soils (pasture, culture) showed higher proportion of fungal pigments

→ Wildfires: selectively destroy quinoid structures (the intensity of spectral maxima decrease)

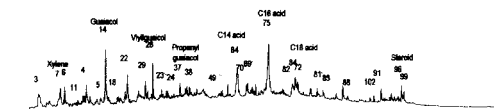


<sup>13</sup>C NMR spectroscopy was used for quantitative determination of the extent of different types of carbons in humic acids

### REPRESENTATIVE CHROMATOGRAMS FROM SOIL LIPID FRACTIONS

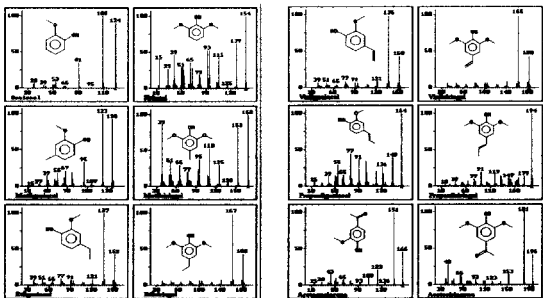


### Overall results from the pyrolysis of humic acids



- The major pyrolytic fragments from the 16 humic acid samples correspond to series of: alkylbenzenes, phenols, alkylphenols, n-alkanes, n-alkenes, fatty acids and steroids
- Concerning alkyl compounds, there were conspicuous series consisting of doublets of alkanes and alkenes in part attributable to the occurrence of structural domains derived from highly resistant biomacromolecules similar to those in cuticles of vascular plants
- The characteristic occurrence of sesquiterpenes and diterpenes was observed in humic acids from soil samples under conifer vegetation

Guaiacyl-type Syringyl-type Guaiacyl-type Syringyl-type



A typical valuable feature of pyrolysis is the release, in the case of lignin-containing materials, of diagnostic signature compounds, "index methoxyphenols" whereas high-temperature oxidative degradation tend to release high yields of benzenecarboxylic acids

Overall results from the NaBO<sub>3</sub> degradation of humic acids

- The major series of degradation compounds were: alkyl (fatty acids, alkanes and alkanedioic acids) and aromatic (phenolic acids and benzenecarboxylic acids)
- There was significant yields of long-chain (>C20) alkanes quite diagnostic for the contribution by vascular plants
- There was a dominance of fatty acids with an even C-number and relatively short (<C20) chain (as could correspond to significant contribution by soil microorganisms)
- The presence of alkanedioic acids could be due to the occurrence of cutans and suberans
- The lignin signature is realized from the presence of phenolic acids. They were accompanied by benzenecarboxylic acids (of a controversial origin, and recently considered as indicators of the impact of fires)
- The occurrence of dehydroabietic acid in humic samples from soils under conifer vegetation was observed

NaBO<sub>3</sub> DEGRADATION PRODUCTS FROM SOIL HAS

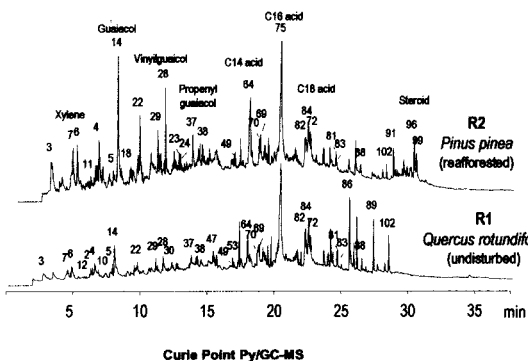
1. <i>n</i> -Propionic acid, dimethyl ester	36. 3,4-Dimethoxybenzoic acid, methyl ester	70. Tetrahydrobenzenecarboxylic acid, dimethyl ester
2. Benzenecarboxylic acid, methyl ester	37. Methylbenzenedicarboxylic acid, dimethyl ester (I)	71. <i>n</i> -Hexadecanoic acid, dimethyl ester
3. Dimethylbenzene	38. Methylbenzenedicarboxylic acid, dimethyl ester (II)	72. Methyl 8,11,15-tridecane-13-oids (dehydroabietate)
4. Methylbenzenecarboxylic acid, methyl ester	39. Methylbenzenedicarboxylic acid, dimethyl ester (III)	73. <i>n</i> -Tricosane
5. <i>n</i> -Butanoic acid, dimethyl ester (succinate)	40. Methylbenzenedicarboxylic acid, dimethyl ester (IV)	74. Furoic acid, methyl ester (marchidate)
6. Benzenecarboxylic acid, methyl ester	41. Methylbenzenedicarboxylic acid, dimethyl ester (V)	75. <i>n</i> -Tetradecane
7. Methylacetone	42. <i>n</i> -Hexadecane	76. Methylbenzenedicarboxylic acid, dimethyl ester (VI)
8. Methylsuccinic acid, dimethyl ester	43. Tetrahydrobenzenecarboxylic acid, methyl ester	77. Hexadecanoic acid, methyl ester
9. <i>n</i> -Pentadecanoic acid, dimethyl ester	44. <i>n</i> -Octadecanoic acid, dimethyl ester (sebacate)	78. <i>n</i> -Pentacosane
10. Methylsuccinylsuccinic acid, dimethyl ester	45. <i>n</i> -Heptadecane	79. Docosanoic acid, methyl ester (beharate)
11. Methylbenzenecarboxylic acid, methyl ester (I)	46. Tetradecanoic acid, methyl ester (myristate)	80. <i>n</i> -Hexacosane
12. Methylbenzenecarboxylic acid, methyl ester (II)	47. Undecanoic acid, dimethyl ester	81. Benzenepentacarboxylic acid, pentamethyl ester
13. Tetrahydrobenzene	48. Hexadecanoic acid, dimethyl ester	82. Tricosanoic acid, methyl ester
14. Oleic acid, dimethyl ester (I)	49. 1,2,3,4-tetrahydrobenzoic acid, dimethyl ester (I)	83. <i>n</i> -Heptacosane
15. <i>n</i> -Hexadecanoic acid, dimethyl ester (I)	50. 1,2,4-tetrahydrobenzoic acid, dimethyl ester (II)	84. Tetraecanoic acid, methyl ester (gigacosanoate)
16. <i>n</i> -Hexadecanoic acid, dimethyl ester (olepate)	51. <i>n</i> -Octadecane	85. <i>n</i> -Eicosane
17. Hydroxyoctanoic acid, methyl ester	52. Dimethylbenzenedicarboxylic acid, dimethyl ester (I)	86. Pentacosanoic acid, methyl ester
18. Methylbenzenecarboxylic acid, methyl ester	53. Dimethylbenzenedicarboxylic acid, dimethyl ester (II)	87. <i>n</i> -Heneicosane
19. <i>n</i> -Tricosane	54. Tetradecanoic acid, methyl ester	88. Hexadecanoic acid, methyl ester (cerotate)
20. Furoic acid, dimethyl ester	55. Pentadecanoic acid, methyl ester	89. <i>n</i> -Triacontane
21. Methylsuccinic acid, dimethyl ester	56. <i>n</i> -Octadecanoic acid, dimethyl ester	90. Heptacosanoic acid, methyl ester
22. <i>n</i> -Hexadecanoic acid, dimethyl ester (paralate)	57. Methylbenzenedicarboxylic acid, dimethyl ester	91. <i>n</i> -Hentriacontane
23. 1,2-Benzene dicarboxylic acid, dimethyl ester (I)	58. Hexadecanoic acid, methyl ester (geranylrate)	92. Octacosanoic acid, methyl ester
24. 1,3-Benzene dicarboxylic acid, dimethyl ester (II)	59. <i>n</i> -Eicosanoic acid, dimethyl ester	
25. Dimethylbenzenedicarboxylic acid, methyl ester (I)	60. Methylbenzenedicarboxylic acid, dimethyl ester (I)	
26. Dimethylbenzenedicarboxylic acid, methyl ester (II)	61. Methylbenzenedicarboxylic acid, dimethyl ester (II)	
27. 1-Methylpyrrolidin-2-ylidenebutanoic acid, d.m.e.	62. Methylbenzenedicarboxylic acid, dimethyl ester (IV)	
28. <i>n</i> -Tetradecane	63. Heptadecanoic acid, methyl ester (myristate)	
29. 1-Methylpyrrolidin-2-ylidenebutanoic acid, d.m.e.	64. <i>n</i> -Hexacosane	
30. Ethene-1,2-diol	65. Octadecanoic acid, methyl ester (stearate)	
31. Methyl-1,2-benzenedicarboxylic acid, d.m.e.	66. 1,2,3,4-tetrahydrobenzoic acid, dimethyl ester (I)	
32. <i>n</i> -Pentadecane	67. 1,2,3,4-tetrahydrobenzoic acid, dimethyl ester (II)	
33. Dodecanoic acid, methyl ester (laurate)	68. 1,2,3,4-tetrahydrobenzoic acid, dimethyl ester (III)	
34. <i>n</i> -Hexadecanoic acid, dimethyl ester (cubate)	69. Hexadecanoic acid, methyl ester	
35. 1,2,3-Propanedicarboxylic acid, dimethyl ester		

NaBO<sub>3</sub> DEGRADATION PRODUCTS FROM SOIL HAS

Compounds	R1	R2	R3	R4	C1	C2	C3	C4	B1	B2	B3	B4	F1	F2	F3	F4
<i>n</i> -Alkanes	—	—	3.9	1.9	—	—	—	—	12.3	2.4	3.1	—	—	—	—	0.8
<i>n</i> -Fatty acids	13.2	15.1	18.4	10.2	8.6	12.5	10.1	7.2	6.9	6.6	11.0	5.8	7.8	7.1	7.6	8.5
Fatty acids	13.4	15.4	18.5	10.2	8.6	12.5	10.2	7.3	6.9	6.7	11.0	5.8	7.8	7.1	7.6	8.5
<i>n</i> - $\alpha$ -Alkanedioic acids	20.0	24.2	16.7	12.2	18.6	12.6	25.2	26.8	14.2	36.7	17.9	24.0	24.7	18.4	19.9	18.9
$\alpha$ -Alkanedioic acids	22.7	27.8	18.3	14.2	21.7	15.0	28.4	30.2	15.7	42.1	20.1	28.3	28.3	19.5	22.1	20.6
Phenolic acids	31.0	18.9	29.3	33.3	25.9	31.5	24.7	25.2	28.2	15.9	29.1	26.6	25.3	21.2	28.8	28.8
Benzenecarboxylic acids	10.9	10.4	14.9	9.3	13.1	21.8	13.0	17.0	17.3	14.1	12.8	11.1	11.8	33.3	21.9	23.9
Total aromatic	46.6	30.7	49.7	47.4	45.8	60.3	46.4	49.6	48.7	38.0	47.1	44.8	47.3	60.7	59.4	57.7
Total aliphatic	43.0	51.2	42.4	30.9	36.8	31.4	45.7	44.1	39.5	58.9	39.4	43.1	44.6	32.0	34.5	33.8

The relative abundances of the major families of perborate degradation compounds showed a good correlation with the signal area measurement in the <sup>13</sup>C NMR spectra

MAJOR PYROLYSIS COMPOUNDS FROM HAS



MOST ABUNDANT PYROLYSIS COMPOUNDS FROM HAS

1. Pentene	27. C5-Allylbenzenes	53. Propenylsyringol	79. Dehydroabietate
2. Hexene	28. Vinylguaiacol	54. Acetylpyrone	80. Kaurane
3. Toluene	29. Ethylguaiacol	55. Tetradecane	81. Eicosane
4. Phenol	30. Syringol	56. <i>n</i> -Tetradecane	82. 9-octadecenoic acid
5. Heptane	31. Undecane	57. Calene	83. <i>n</i> -Eicosane
6. Styrene	32. C2-Allylthiophalenes	58. Longiphen	84. Octadecanoic acid
7. Xylene	33. <i>n</i> -Undecane	59. Pentadecane	85. Heneicosane
8. Creosol	34. C3-Allylindanes	60. Syringylactone	86. <i>n</i> -Heneicosane
9. Octane	35. C2-Allylbenzenes	61. <i>n</i> -Pentadecane	87. Docosane
10. Indane	36. C3-Allylthiophenol	62. Hexadecane	88. <i>n</i> -Docosane
11. C5-Allylbenzenes	37. Propenylguaiacol	63. Heptadecane	89. <i>n</i> -Tricosane
12. Vinylthiophenol	38. Acetylguaiacol	64. Tetradecanoic acid	90. <i>n</i> -Tetradecane
13. C2-Allylthiophenols	39. Propylguaiacol	65. Resene	91. <i>n</i> -Pentacosane
14. Guaiacol	40. Octadecane	66. 1-(1-hydro- <i>n</i> -ethylphenyl)ethane	92. <i>n</i> -Hexacosane
15. Nonane	41. Methylsyringol	67. Heptadecane	93. Steroid
16. Naphthalene	42. C3-Allylthiophalenes	68. <i>n</i> -Heptadecane	94. Steroid
17. <i>n</i> -Nonane	43. <i>n</i> -Dodecane	69. anteiso-C15 fatty acid	95. Steroid
18. Methylindanes	44. C2-Allylthiophalenoils	70. Iso-C15 fatty acid	96. Steroid
19. Tetralin	45. C3-Allylthiophenols	71. Pentadecanoic acid	97. Steroid
20. C4-Allylbenzenes	46. Guaiacylacetone	72. Octadecane	98. Steroid
21. C3-Allylthiophenols	47. Vinylsyringol	73. Norabietalene	99. Steroid
22. Methylguaiacol	48. Ethylsyringol	74. <i>n</i> -Octadecane	100. Steroid
23. Decane	49. <i>n</i> -Dodecane	75. Hexadecanoic acid	
24. C1-Allylthiophalenes	50. <i>n</i> -Tetradecane	76. C10-Allyltylhydroindene	
25. <i>n</i> -Decane	51. C3-Allylthiophalenoils	77. Nonadecane	
26. C2-Allylindanes	52. C4-Tetralins	78. <i>n</i> -Nonadecane	

(*N*-compounds, polycyclic compounds are more frequent after pyrolysis than after perborate chemolysis)

(Steroids were more resistant than diterpene resin acids to pyrolytical treatment)

## TOTAL ABUNDANCES OF THE DIFFERENT GROUPS OF COMPOUNDS IDENTIFIED BY PYROLYSIS

	R1	R2	R3	R4	C1	C2	C3	C4	B1	B2	B3	B4	F1	F2	F3	F4
Phenols	6.93	11.18	6.23	9.54	22.32	7.12	19.93	15.72	11.56	15.06	18.68	12.78	20.06	11.49	5.37	7.98
Alkylbenzenes	2.89	6.08	4.36	3.61	9.10	3.15	18.05	2.73	5.40	4.74	6.80	1.35	6.16	5.33	2.16	4.88
Naphthalenes	0.40	1.07	0.53	1.06	1.36	0.19	0.51	0.57	1.54	0.73	1.36	—	1.32	2.89	0.76	0.92
Naphthalenones	—	—	—	—	—	—	—	—	—	—	—	—	0.17	—	0.20	0.73
Indenes	0.68	0.77	0.42	1.08	1.21	0.37	0.36	0.71	1.04	1.01	1.73	—	2.54	0.87	5.61	1.02
Tetralins	—	—	—	0.63	—	—	—	—	—	—	—	—	0.76	—	0.04	0.21
Guaiacols	4.59	7.55	4.49	6.37	12.24	3.98	9.54	8.67	7.48	7.15	9.95	9.85	15.51	4.00	4.16	2.94
Syringols	1.08	0.46	0.83	—	7.42	1.22	5.34	4.27	1.61	4.36	3.25	3.13	—	—	—	0.86
Methoxyphenols	5.61	7.90	5.32	6.25	19.22	5.16	14.12	12.43	8.93	11.41	12.93	12.78	15.38	4.00	4.16	3.56
Steroids	1.33	3.52	3.38	1.60	—	—	0.22	—	4.28	0.87	—	—	1.51	10.06	1.90	—
Alkanes	9.76	2.85	7.16	3.70	2.58	1.04	3.03	5.08	3.53	4.81	5.40	0.66	0.05	3.10	3.36	2.40
Alkenes	4.26	1.55	2.66	4.65	2.11	0.72	3.34	2.85	5.08	4.31	3.25	0.78	0.18	5.71	3.69	1.55
Fatty acids	17.33	11.91	10.77	15.53	4.68	1.16	3.85	6.69	10.79	7.22	7.78	—	7.18	0.57	4.86	5.56

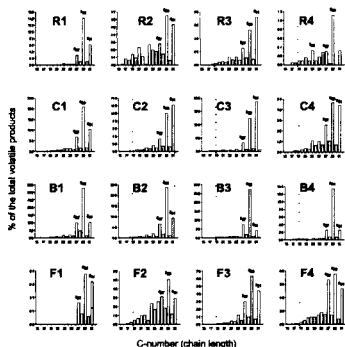
In most samples, fatty acids were the most abundant alkyl pyrolysis compounds (except, e.g., in soil after high-intensity fire). The release of cycloalkanes and cycloalkenes was characteristic of pyrolysis (tetralins, indenes...) whereas hydroaromatic compounds were infrequent after perborate degradation.

## RELATIVE YIELDS OF NaBO<sub>3</sub> AND PYROLYSIS DEGRADATION PRODUCTS FROM HAs

	R1	R2	R3	R4	C1	C2	C3	C4	B1	B2	B3	B4	F1	F2	F3	F4
Total n-alkanes	—	—	3.9	1.9	—	—	—	—	12.3	2.4	3.1	—	—	—	—	0.8
	9.8	2.8	7.3	3.7	2.6	1.1	3.3	5.1	3.5	4.8	5.4	0.7	0.1	3.1	3.3	2.4
Total n-fatty acids	13.4	15.4	16.5	10.2	8.6	12.5	10.2	7.3	6.9	6.7	11.0	5.8	7.8	7.1	7.6	8.5
	17.3	11.9	10.8	15.5	4.7	1.2	3.8	6.7	10.8	7.2	7.8	—	7.2	0.6	4.9	5.6
Total $\alpha,\omega$ -alkanedioic	22.7	27.9	18.3	14.2	21.7	15.0	29.4	30.2	15.7	42.1	20.1	28.3	28.3	19.5	22.1	20.8
Total n-alkenes	4.3	1.5	62.6	4.6	2.1	0.7	3.3	2.8	5.1	4.3	3.2	0.8	0.2	5.7	3.7	1.6

Wet chemical degradation with NaBO<sub>3</sub> did not release a substantial amount of hydrocarbons. Compared to the pyrolytic alkanes, the perborate-released alkanes often show a shifting of the maxima towards the lowest molecular weight (> C<sub>20</sub> alkanes). This suggests that pyrolysis is more efficient than chemolysis in the case of alkanes because the rapid thermoevaporation probably prevents chain breakdowns releasing entrapped molecules. In general the relative yield of total alkyl compounds was higher with chemolysis than with pyrolysis.

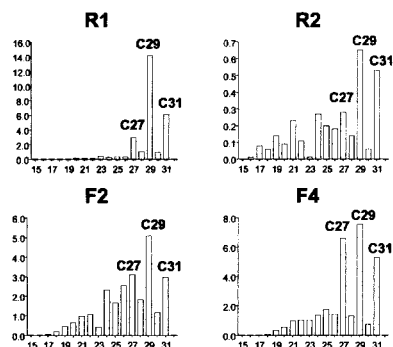
## n-ALKANES DISTRIBUTION IN THE SOIL LIPID FRACTIONS



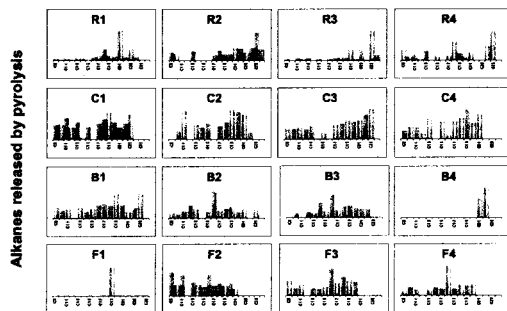
The homologue series of free alkanes (direct solvent extraction) showed typical long-chain (>C<sub>20</sub>) and odd-to-even C number preference typical of epicuticular waxes of vascular plants. Both features are smoothed in alkyl compounds released after thermal wet chemical degradations as expected from:

- chain breakdown during the degradation reactions and
- A natural preferential association of comparatively short-chain alkyl molecules into the macromolecular structures of the humic acids.

## n-ALKANES DISTRIBUTION IN THE SOIL LIPID FRACTIONS

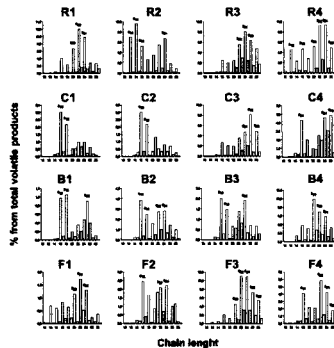


The occurrence of relatively short-chain (<C<sub>20</sub>) alkanes is often considered to be due to microbial metabolism (mainly when there is no high odd/even C-number preference), but this fact to large extent depend on vegetation type (R2= pine forest) and of the effect of wildfires (F2, F4)



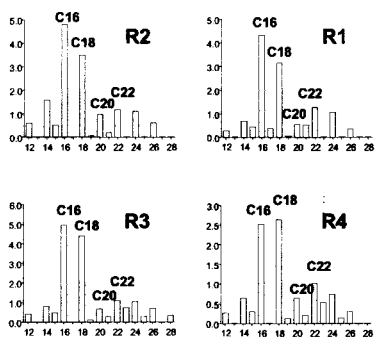
Analytical pyrolysis of humic acids yields a conspicuous C9-C25 series of alkanes, in most cases with the maximum ca. C21. With the exception of some cases in which a clear even-to-odd C-number preference is observed (in particular, in well-preserved oak forests) in the other alkane series the C preference index and their relative chain length indicated that the major constituents have not a recent biogenic origin (i.e., epicuticular waxes of higher plants) but have been probably subjected to intense microbial and abiotic reworking leading to short-chain homologues.

## n-FATTY ACIDS DISTRIBUTION IN THE SOIL LIPID FRACTIONS



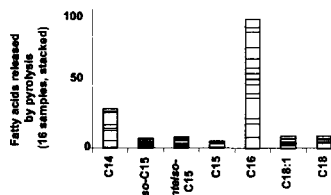
The homologue series of free (solvent-extracted) fatty acids showed a wide range (C<sub>7</sub>-C<sub>29</sub>) indicating coexistence of microbial acids and acids from higher-plant waxes

## n-FATTY ACIDS DISTRIBUTION AFTER NaBO<sub>3</sub> DEGRADATION



As usual in most wet chemical degradation procedures, perborate degradation released homogeneous C<sub>12</sub>-C<sub>28</sub> series of fatty acids where palmitic and stearic acids were the dominant compounds.

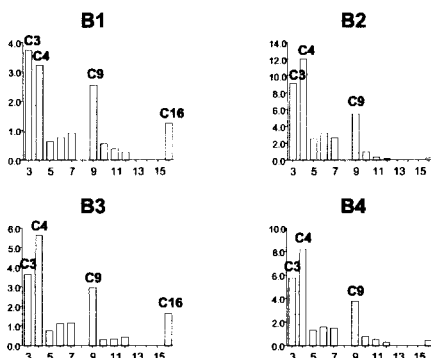
## Homogeneous patterns of fatty acids obtained by Curie-point pyrolysis



Pyrolysis yielded narrow fatty acid series (mainly C12-C18) with maximum at the C16 acid, whereas wet chemical degradation yielded a conspicuous C12-C28 fatty acid series with maximum at C16 or C18. Diagnostic *iso*- and *anteiso*-fatty acids (mainly C15) are detected by both chemical and thermal degradation methods.

Nevertheless, wet chemical degradation yielded additional series of  $\alpha,\omega$ -alkanedioic acids (up to 40% of the total degradation compounds) including relatively long-chain molecules (>C9 and frequently >C12 acids) with characteristic bimodal distribution suggesting specific breakdowns at the level of C9 and C4 in-chain substitutions of the most frequent biogenic polyunsaturated fatty acids and their hydroxy-derivatives such as the precursors of cutans, suberans or other natural polyesters.

## $\alpha,\omega$ -ALKANEDIOIC ACIDS DISTRIBUTION AFTER NaBO<sub>3</sub> DEGRADATION



## Final considerations (I)

### General:

As a whole, the relative yields of alkyl compounds were higher after chemolysis than after pyrolysis.

N-compounds and polycyclic molecules were more frequent after pyrolysis than after NaBO<sub>3</sub> degradation.

After pyrolysis, the occurrence of sesquiterpenes and diterpenes was observed in humic acids from soils under conifer vegetation. Steroids were more resistant than diterpene resin acids to pyrolytical treatment. Several diterpene resin also survives NaBO<sub>3</sub> degradation.

### Alkyl products:

In most samples, fatty acids were the most abundant alkyl pyrolysis products (except, e.g., in soil after high-intensity fire). The release of cycloalkanes and cycloalkenes was characteristic of pyrolysis (tetralins, indenes...) whereas hydroaromatic compounds were unfrquent after NaBO<sub>3</sub> degradation.

Pyrolysis yielded narrow fatty acid series (mainly C12-C18) with maximum at the C16 acid, whereas NaBO<sub>3</sub> degradation yielded richer C12-C28 fatty acid series with maximum at C16 or C18. Diagnostic *iso*- and *anteiso*-fatty acids (mainly C15) are detected by both chemical and thermal degradation methods.

## Final considerations (II)

The NaBO<sub>3</sub> degradation yielded  $\alpha,\omega$ -alkanedioic acids (up to 40% of total compounds) with characteristic distribution suggesting specific breakdowns at the level of C9 and C4 in-chain substitutions of the most frequent polyunsaturated fatty acids and their hydroxy-derivatives (cutans, suberans or natural polyesters). Conversely, pyrolysis releases unspecific series of alkanes with comparatively lower yields.

Both degradation procedures produces more or less intense alteration of labile aliphatic molecules, yielding a biased information about the original alkyl constituents in the humic acid. Our results suggest that pyrolysis is a more accurate technique than chemolysis for the study of alkanes. The rapid thermoevaporation probably prevents chain breakdowns, and releases entrapped molecules.

Based on indirect evidences (i.e., that pyrolysis lead to a non-degraded charred residue whereas NaBO<sub>3</sub> degradation completely solubilized the humic acid sample) it is postulated that the latter technique produces a less intense alteration of the lightly-bonded moiety of the alkyl domain in humic acid macromolecules. On the other hand, secondary endothermic condensation, or "thermal fixation" of alkyl compounds would limit the pyrolytic information in the form of volatile products.

Compared to soil lipid, both degradation methods lead to shorter chain-length compounds as a probable effect of: i) breakdown during the degradation reactions and, ii) preferential association of comparatively short-chain alkyl molecules into the macromolecular structures of the humic acids.