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Chemistry and Ecology

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713455114>

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Online publication date: 30 September 2010

To cite this Article Piedra Buena, A. , Almendros, G. , González-Vila, F. J. , González-Pérez, J. A. and Knicker, H.(2010) 'Transformations in carbon and nitrogen-forms in peat subjected to progressive thermal stress as revealed by analytical pyrolysis', *Chemistry and Ecology*, 26: 5, 361 – 370

To link to this Article: DOI: 10.1080/02757540.2010.504665

URL: <http://dx.doi.org/10.1080/02757540.2010.504665>

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Transformations in carbon and nitrogen-forms in peat subjected to progressive thermal stress as revealed by analytical pyrolysis

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(Received 16 November 2009; final version received 17 May 2010)

To study the characteristic N-forms of humic-type materials, samples of sapric peat from Galicia (northern Spain) were heated at 350 °C for 60, 90, 120, 150 and 180 s, and studied by Curie-point Py-GC/MS, solid-state CPMAS ¹³C-NMR and ¹⁵N-NMR spectroscopies. NMR analysis of the peat samples in the progressive heating stages showed the concentration of heterocyclic N-forms, the maximum structural transition amide-to-heterocyclic forms being observed in samples heated for 120 s (56% heterocyclic N and 34% aromatic C). Under more drastic conditions all N-forms were depleted. Correlation between spectroscopic and pyrolytic data betrayed specific pyrolytic markers for the different N-forms. The intensity of the ¹⁵N-NMR amide peak tended to be positively correlated to the yield of indoles, imidazoles and pyrazoles, and negatively correlated to those of benzonitriles and pyrazines. Analytical pyrolysis also showed a progressive enrichment in lipids and alkyl macromolecules with increasing heating intensity, and a decrease in lignin-derived, polysaccharide-derived and N-containing compounds. The relative abundance of non-methoxyphenolic aromatic compounds did not change. The N-compounds in peat samples unheated or heated for 60–90 s released methylpyrazole, dimethylpyrroline, methylidiphenylindole and pyrazole, whereas peat samples heated for 120 s mainly yielded methylpyrazine and methylidiphenylindole. Dimethylpyrroline and pyrazine prevailed in samples heated for 150 s, whereas samples heated for 180 s yielded mainly pyrrole. Pyrolysis data presented low possibilities for forecasting the extent of the O-alkyl domain, but reflected quantitatively the transformations in the lignin-like moiety. Both techniques coincide in pointing out the accumulation of a recalcitrant alkyl domain possibly derived from abiotic condensations or inherited lipid biomacromolecules.

Keywords: humification pathways; N-compounds; fire effects; controlled heating

1. Introduction

Despite the potential of current analytical methods to identify the chemical constituents of humic-type materials, to date ~50% of their N consists of unknown structures. Whereas most classical studies postulated that large amounts of N-compounds in soil organic matter consist of heterocyclic compounds [1], these studies were mainly based on indirect evidence, i.e. the typical fact that only a limited portion of N-structures in soil humic substances yielded amino acids after 6 M HCl hydrolysis [2].

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Further studies based on ^{15}N nuclear magnetic resonance (NMR) evidenced the practical lack of heterocyclic N-compounds in most natural soil samples [3], including humus types with large maturity. Experiments based on ^{15}N -labelled lignocelulosic biomass demonstrated that thermal processes (i.e. laboratory heating, wildfires or geothermal processes) on soils and sediments were the most successful in generating non-amide N components producing intense ^{15}N -NMR signals in the region of heterocyclic N-compounds [4].

Compared with the poor resolution of the broadband NMR spectra of macromolecular substances, analytical pyrolysis combined with gas chromatography and mass spectrometry (Py-GC/MS) is a powerful complementary tool for the structural characterisation of humic substances. Owing to the high stability of the intramacromolecular links in recalcitrant humic matter, their breakdown requires considerable energy. The disadvantage of this technique consists, in general, of the formation of artefacts (or secondary compounds) and the high yields of cleavage products with very low molecular masses, which are not valid diagnostic fragments for structural analysis. However, most of these secondary compounds have been mainly reported for polysaccharides and alkanes, not for N-compounds. Also, for the reliable interpretation of valid 'pyrolytic fingerprints' from humic-type substances, previously characterised humic reference material is often used.

In this study, a sample of sapric peat was subjected to heating under laboratory conditions comparable with those in natural wildfires or controlled burnings [5]. This peat was then analysed by ^{13}C -NMR and ^{15}N -NMR for quantitative assessment of the different N-forms which could be generated in the course of the heating experiment. The results were compared with the yields of the different N-compounds observed by analytical pyrolysis. This experimental approach should be valid to identify surrogate indicators of the degree of diagenetic transformation of amide N in soils and sediments, rather than establishing straightforward cause-to-effect relationships between the percentages of different N forms, as seen using analytical pyrolysis and ^{15}N -NMR.

In consequence, the aim of this research was to infer some hypotheses about fire-induced changes in the structural characteristics of the stabilised N domain in humic substances. This would help clarify specific questions related to the interpretation of the assemblages of pyrolysis compounds, for example:

- (1) comparison of the relative abundances of pyrolysis compounds with results obtained using CPMAS ^{13}C -NMR;
- (2) identification of possible signature pyrolysis N-compounds for monitoring thermal impacts in humic matter;
- (3) quantification of the relative abundances of the major families of C and N pyrolysis compounds to differentiate between those derived from thermolabile structures and those derived from thermostable (or newly formed) structures;
- (4) comparison of pyrolysis patterns after relatively mild or severe heating, to discuss the occurrence of successive stages; and
- (5) the thermal stabilisation of the alkyl structures, providing some supportive data on the postulated existence of not only a polycyclic aromatic, but also a recalcitrant alkyl domain.

2. Materials and methods

2.1. Humic preparations

Peat material was collected from a sapric basement at Buyo (Galicia, north-western Spain), taken as a composite average sample from the upper 100 cm of the soil. The elementary composition of peat (ash-free) was $56.7\text{ g kg}^{-1}\text{C}$, $6.2\text{ g kg}^{-1}\text{H}$, $1.6\text{ g kg}^{-1}\text{N}$ and $35.4\text{ g kg}^{-1}\text{O}$, with a C/N ratio

(w/w) of 35.0. Other analytical characteristics and spectroscopic ^{13}C - and ^{15}N -NMR data have been reported elsewhere [4,6].

Peat samples were homogenised by air milling, sieved to 500 μm and subjected to dry heating according to the experimental conditions previously described [5,7]. Samples were then placed in porcelain crucibles and subjected to controlled isothermal heating at 350 $^{\circ}\text{C}$ for progressive periods (60, 90, 120, 150 and 180 s) in an electric furnace (Wosthoff o.H.G, Bochum) operating at atmospheric pressure and under a 625 $\text{mL} \cdot \text{min}^{-1}$ suction flow.

2.2. Solid-state CPMAS NMR spectroscopy

Solid-state ^{13}C -NMR spectra were obtained using the cross-polarisation (CP) and magic angle spinning (MAS) technique with a Bruker DSX 200 spectrometer operating at a frequency of 50.32 MHz, with a zirconium rotor of 7 mm outer diameter with KEL-F caps. The acquisition conditions were those considered to provide reliable quantitative integration values in the different spectral regions [8]. MAS was performed at 6.8 kHz. The 90° ^1H -pulse width was 5.4 μs , and the contact time was 1 ms. Between 5000 and 8000 scans were accumulated using a pulse delay of 300 ms. Tetramethylsilane (= 0 ppm) was used to calibrate the chemical shift range. Prior to Fourier transformation, a line broadening of 50 Hz was applied. The intensity distribution of the carbon atoms in different structures was determined by integration of signal intensity in various chemical shift regions using the instrument software.

Solid-state ^{15}N CPMAS NMR spectra were acquired on a Bruker DMX 400 instrument operating at 40.56 MHz and applying a contact time of 1 ms. A 90° pulse width of 5.8 μs , a pulse delay of 150 ms and a line broadening of 100 and 150 Hz were applied. Between 5 and 7×10^5 scans were accumulated at a MAS speed of 5.5 kHz. The chemical shifts were standardised to the nitromethane scale (0 ppm) and adjusted with ^{15}N -labelled glycine (-347.6 ppm).

2.3. Curie-point pyrolysis/GC-MS

Pyrolysis was carried out in a Curie-point pyrolyser (Horizon Instruments) attached to a Varian Saturn 2000 GC-MS system. The samples were heated at 510 $^{\circ}\text{C}$ Curie-point temperature for 5 s on ferromagnetic wires. The interface temperature was 250 $^{\circ}\text{C}$. The liquid CO_2 cryogenic unit was programmed from -30°C (1 min) to 300 $^{\circ}\text{C}$ at 20 $^{\circ}\text{C} \cdot \text{min}^{-1}$, and the GC oven was adjusted from 50 to 100 $^{\circ}\text{C}$ at 32 $^{\circ}\text{C} \cdot \text{min}^{-1}$, and then to 320 $^{\circ}\text{C}$ at a rate of 6 $^{\circ}\text{C} \cdot \text{min}^{-1}$. For the chromatographic analysis, a fused-silica capillary column coated with CPSil (film thickness 0.4 μm) and He as carrier gas (25 m \times 0.32 mm) were used. Compound identification was based on literature data and further comparisons with mass spectral libraries [9,10].

In order to present an index that could be related to the extent to which the complexity of the pyrolytic assemblages increased or decreased in terms of the thermal impact, the Shannon diversity index [11,12] was calculated for the identified compounds. This index was selected as a conventional measurement of the selective depletion or, conversely, the presence of newly formed compounds, generated by endothermal reactions which probably concur at the different heating stages.

3. Results and discussion

3.1. ^{13}C -NMR spectra

Figure 1 shows the CP/MAS ^{13}C - and ^{15}N -NMR spectra of the peat samples subjected to heating at 350 $^{\circ}\text{C}$ for 0 and 120 s. The major spectral regions, as well as the main signals in

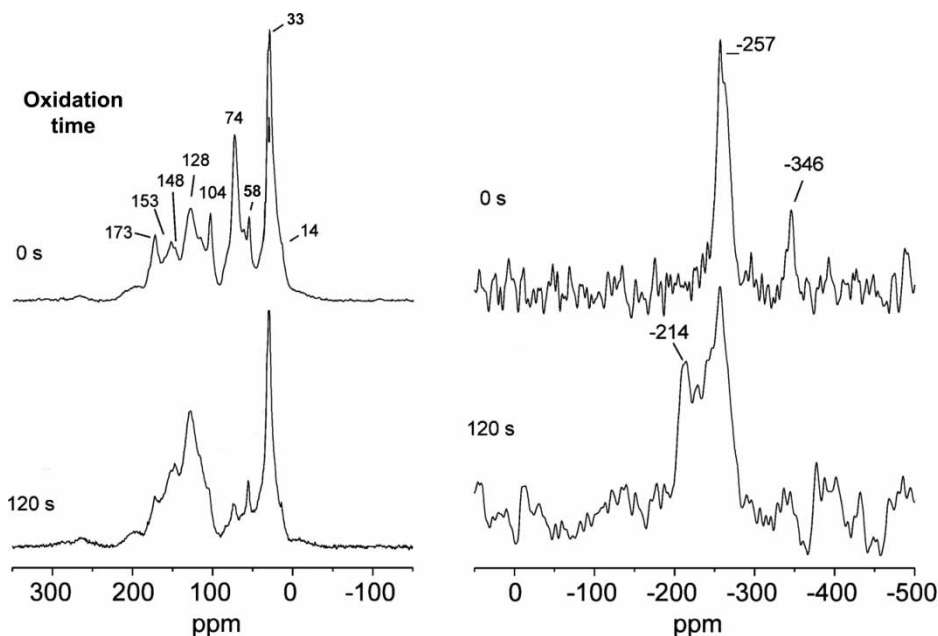


Figure 1. ^{13}C (left) and ^{15}N (right) NMR spectra from peat samples subjected to 0 and 120 s of heating at 350 °C.

the ^{13}C spectra, were interpreted according to standard assignments [13]: 0–46 ppm = alkyl (13 = methyl, 21 = acetate, 30 = polymethylene); 46–110 ppm = *O*-alkyl (56 = methoxyl, 73 = carbohydrate-derived); 110–160 ppm = unsaturated aromatic (126 = H-substituted, 147 = *O*- or *N*-substituted); 160–200 ppm = C=O (172 = COOH, 198 = ketone/aldehyde).

As expected, the ^{13}C -NMR spectra clearly showed that heating led to almost total depletion of the *O*-containing functional groups, i.e. decarboxylation and dehydration [14]. This was evidenced by the decreased intensity of both carboxyl (173 ppm) and methoxyl (58 ppm) signals in the sample heated for 120 s. Nevertheless, a significant amount of alkyl carbon (30 ppm) presented a thermal stability similar to that of the residual aromatic carbon (128 ppm).

3.2. ^{15}N -NMR spectra

Peak assignments for the solid-state ^{15}N -NMR spectra (referenced to nitromethane = 0 ppm) were carried out according to Kögel-Knabner [15]: 25 to –25 ppm = nitrate, nitrite and nitro; –25 to –90 ppm = imine, phenazine, pyridine and Schiff bases; –90 to –145 ppm = purine and nitrile; –145 to –220 ppm = chlorophyll-N, purine/pyrimidine, imidazole and substituted pyrroles; –220 to –285 ppm = amide/peptide, *N*-acetyl derivatives of amino sugars, tryptophane, proline, lactams, unsubstituted pyrroles, indoles and carbazoles; –285 to –325 ppm = NH in guanidine, NH_2 - and NR_2 -groups (N_δ -arginine and N_α -citrulline, N_ϵ -arginine N_ω -citrulline), urea, nucleic acids and aniline derivatives; –325 to –350 ppm = free amino groups in amino acids and amino sugars; –350 to –375 ppm = NH_4 .

As shown in Figure 1, progressive concentration of heterocyclic *N*-forms (peak centred at –214 ppm) was evident in the ^{15}N -NMR spectra in the course of heating. The transition from amide to heterocyclic forms was observed in samples heated for 120 s, whereas under more drastic conditions (150–180 s heating) both amide and heterocyclic *N*-forms were depleted.

3.3. Curie-point pyrolysis/GC-MS

The molecular assemblages analysed by Py-GC/MS suggested progressive enrichment in the yields of alkyl compounds during the course of heating, with a concomitant decrease in the concentration of methoxyphenols, carbohydrate-derived and N-containing products, whereas the yields of non-methoxyphenolic aromatic compounds remained relatively constant (Table 1 and Figure 2). The decrease in the yields of N-containing compounds with increasing length of thermal treatment was also observed in an experiment with whole soil subjected to 500 °C for 3 and 9.9 s [16]. Also, these authors observed that increasing the temperature from 300 to 500 °C, and then to 700 °C for 3 s did not lead to substantial differences in the identified N-compounds or in their relative ion intensities. Nevertheless, it must be pointed out that peat and whole soil may respond differently to heating. In fact, in whole soil the effect of the mineral matrix leads, for example, to aromatisation of humic acids, generating products such as alkylbenzenes and polycyclic aromatic hydrocarbons [17]. However, in the case of N-compounds, some authors have concluded that

Table 1. N-containing compounds identified by Curie-point Py-GC/MS in sapric peat samples heated for 0–180 s at 350 °C (total abundances calculated from total ion chromatographic area expressed as percentages of total N-containing compounds).

Compound	Heating time (s)					
	0	60	90	120	150	180
Pyrazole	0	0	32	0	0	0
Methylpyrazole	20	21	17	0	0	0
Methylpyrazine	0	0	0	49	0	0
Methylpyrroline	9	0	0	0	0	0
Methylpyrrole I	0	0	0	5	6	0
Pyrrole	0	1	1	1	0	95
Dimethylpyrroline I	18	0	0	0	41	0
Pyrrolidine	0	0	2	0	0	0
Pyridine	0	1	1	3	0	0
Pyrrole methanol	2	0	0	0	0	0
Methylpyrrolidine	13	0	0	0	0	0
Pyrazine	0	0	0	0	19	0
Dimethylpyrazole	0	0	5	0	0	0
Dimethylimidazole	0	3	2	0	0	0
Imidazolometanol	0	16	9	6	6	0
Trimethylpyrrole	0	0	0	0	4	0
1 <i>H</i> -Pyrrole-ethyl-methyl-	0	0	2	0	0	0
Dimethylpyrazole	2	0	0	0	4	0
2,5-Pyrrolidinedione	2	5	3	0	0	0
Hydroxybenzotrile	0	0	0	1	0	0
Trimethylpyrazole	0	0	4	0	0	0
Methoxypyrazine	7	0	0	0	0	0
Methylpyrimidone	0	8	0	0	0	0
2-(1 <i>H</i>)Pyridinone, 1-methyl	0	0	0	2	0	0
Benzeneacetonitrile	0	0	0	0	4	0
Indolizine	0	0	0	0	0	5
Pyrrolidinone	0	0	1	0	0	0
Indole	0	1	0	0	0	0
Dimethylpyridine	0	2	3	9	0	0
1 <i>H</i> -Pyrrole, 3-ethyl, 2,4-dimethyl-	0	0	0	3	3	0
Trimethylindole	0	0	0	0	3	0
Dimethylindole	6	15	8	4	0	0
Dimethoxypyrimidine	0	0	2	4	0	0
Pyrolizine-1-methanol, hexahydro-(1 <i>S</i> - <i>cis</i>)	0	2	1	0	0	0
Methyldiphenylindole	20	26	8	14	7	0
1 <i>H</i> -Isoindole-1,3(2 <i>H</i>) dione,2-(1,1'-biphenyl)-4-yl-	0	0	0	0	4	0

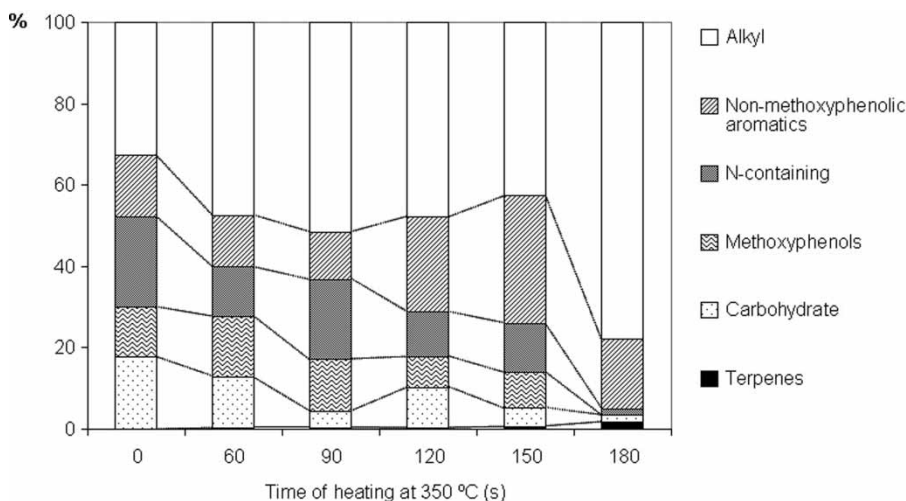


Figure 2. Kinetics of the release of pyrolytic compounds from peat samples subjected to progressive heating (0–180 s) at 350 °C. Semiquantitative data correspond to peak area integration in the gas chromatograms calculated as percentages of the total volatile compounds.

heterocyclic nitrogenated compounds are indeed significant components of the soil organic matter (SOM), rather than degradation products of other molecules due to pyrolysis [18].

It is worth pointing out that the severely heated sample (> 150 s at 350 °C) showed a dominant, thermal-resistant alkyl domain, as revealed both by ^{13}C -NMR and Py-GC/MS (Table 1, Figures 1 and 3). Such charred materials, which would be *a priori* ascribed to black-carbon consisting of graphite-like polynuclear structure, in fact seem to consist of a heterogeneous protokerogen-like matrix including resistant polyalkyl or cycloalkyl structures, in addition to a significant domain of ‘black-nitrogen’ structures [19], which after pyrolysis yields mainly pyrazine, dimethylpyrroline and pyrrole.

Concerning the nature of the individual N-compounds, the Py-GC/MS results indicated close dependence on temperature: peat samples unheated or heated at 350 °C for 60–90 s consisted mainly of methylpyrazole, dimethylpyrroline, methyl-diphenylindole and pyrazole, whereas peat samples heated for 120 s mainly yielded methylpyrazine and methyl-diphenylindole (Table 1 and Figure 3). Dimethylpyrroline and pyrazine prevailed in samples heated for 150 s, whereas pyrrole and phthalic acid, 3-nitro (1,2-benzenedicarboxylic acid, 3-nitro) accounted for ~97% of the N-containing pyrolysis compound in samples heated for 180 s (Table 1). These changes in the relative yields of the released compounds suggest that at low heating intensities the identified compounds are originated mainly from amino acids (pyrazole, methylpyrazole, methyl-diphenylindole) and other as yet not elucidated sources, either from plants, microorganisms or soil [18,19]. With increasing heating time (samples heated for 120 and 150 s), some compounds from hydroxyacids, dipeptides and polypeptides from soil, plant or microbial origin (pyrazine, methylpyrroline) were released [18,19]. At 180 s heating, only pyrrole or N-compounds typical of soils (phthalic acid, 3-nitro) have been identified [16,18,19,20]. Schulten et al. [16] indicate that the identification of long-chain alkylnitriles is usually obscured by olefins, which have similar retention times.

The intensity of the ^{15}N -NMR amide peak was positively associated with the yield of indoles, imidazoles and pyrazoles, and negatively correlated with benzonitriles and pyrazines. Consequently, these compounds could act as surrogate indicators of the extent of the heterocyclic-N domain in the peat under study, although it is clear that the release of heterocyclic N-pyrolysis

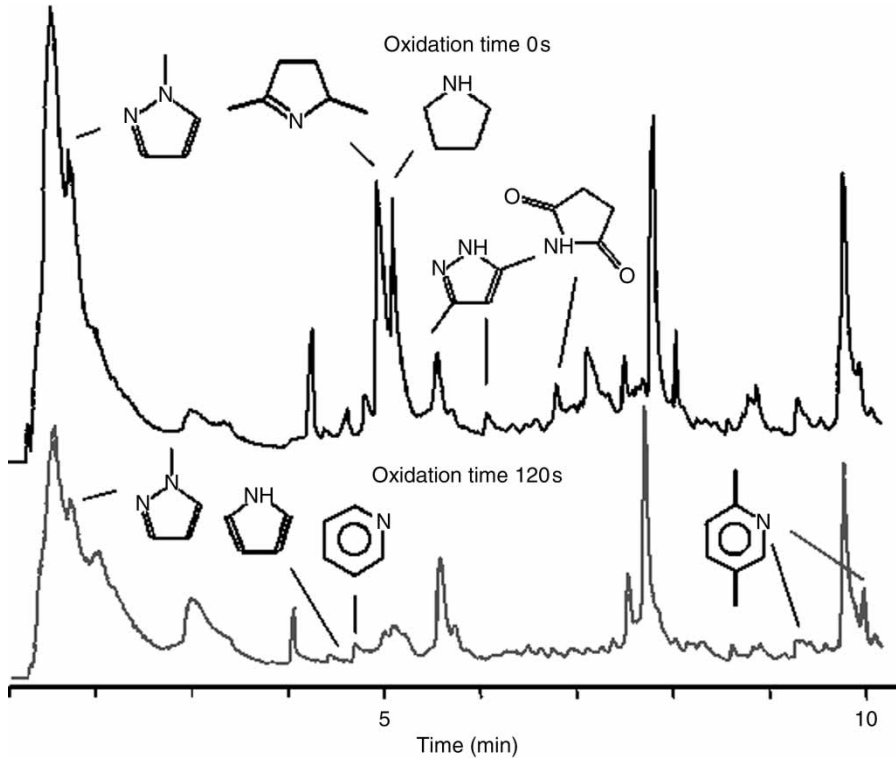


Figure 3. Chromatographic separation range (0–10 min) of pyrolysis compounds from peat samples subjected to heating at 350 °C for 0 and 120 s. The structures of the major compounds are shown on the corresponding peaks.

compounds does not necessarily mean that such structures were present in the original material. In fact, some authors have reported that pyrolysis may lead to the formation of alkylated benzenes and naphthalenes from fatty acids [21,22], as well as to thermal degradation of the backbone of polysaccharides and proteins through secondary reactions [23].

The results of the Shannon diversity indices [11,12] calculated for each group of pyrolysis products are presented in Figure 4. In the case of the non-methoxyphenolic aromatics, carbohydrate-derived compounds and the whole set of identified compounds, the indices showed no linear relationship with the time of heating, with two peaks or maxima at ~ 90 and 150 s of heating. The calculated Shannon diversity index for N-compounds showed a similar level in unheated samples and samples heated for 60–150 s, with a maximum at 90 s of heating, whereas in samples heated for 180 s, the diversity value dramatically decreased. Similar behaviour was observed for methoxyphenols, where the diversity index increased from 0 to 90 s of heating, then decreased from 90 to 150 s of heating; no methoxyphenols were detected in the peat sample heated for 180 s. The alkyl compounds (alkanes, fatty acids and olefins) showed very similar trends. The decreased diversity in samples heated from 0 to 150 s may correspond to the decrease observed in the concentration of several compounds, interpreted as being due to active thermoevaporation. At high temperatures (180 s heating), the increased diversity seems to be related to the occurrence of new short-chain alkyl compounds, mainly olefins, resulting from the thermal breakdown of heat-resistant polymethylene molecules (Figure 5). A depletion of odd-numbered with respect to even-numbered alkanes and fatty acids was also observed.

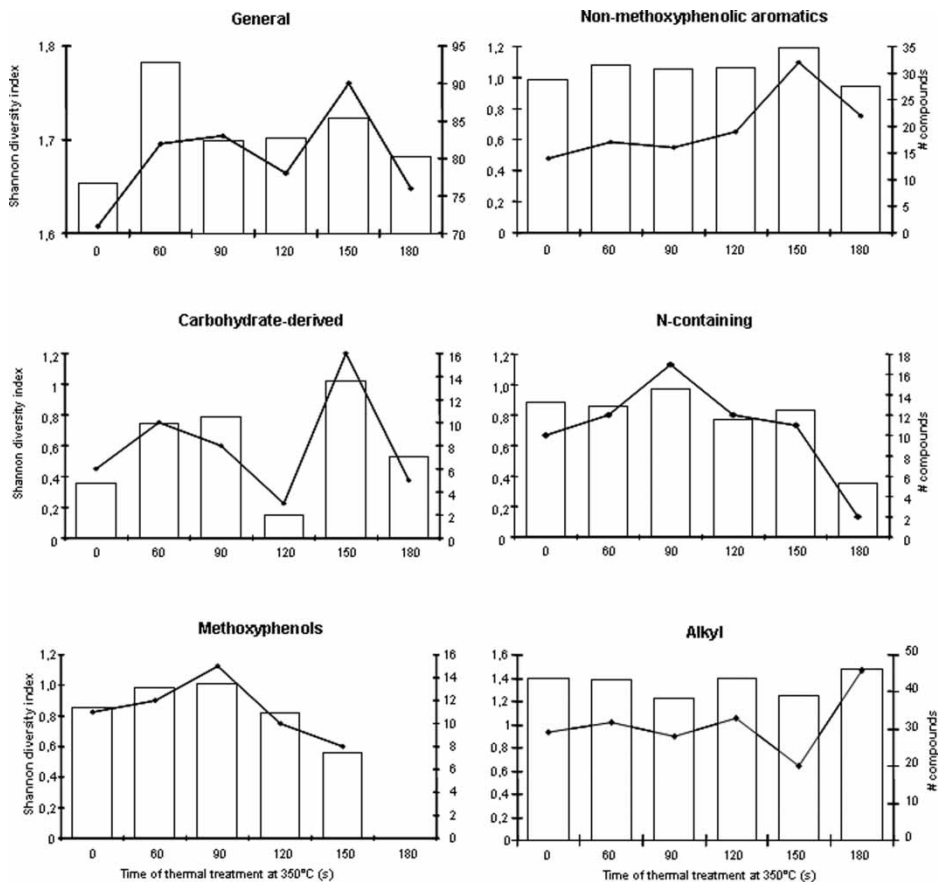


Figure 4. Evolution of possible newly formed chemical structures during the course of isothermal heating (350 °C) measured as Shannon diversity indices (bars) and number of compounds (line series). General: indicates the molecular assemblages of the whole set of identified pyrolytic compounds.

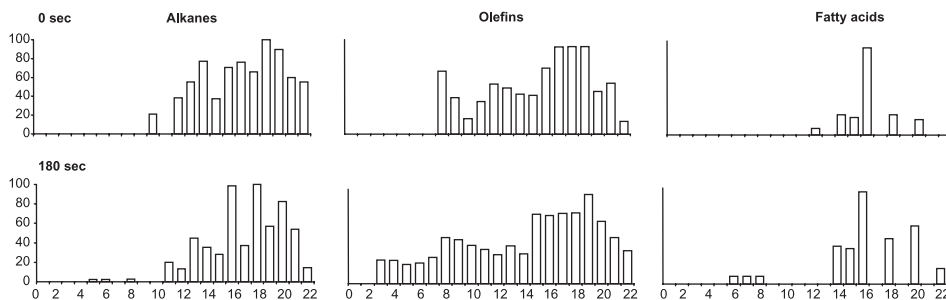


Figure 5. Relative abundance vs. number of atoms in the carbon chain of the series of alkyl compounds: alkanes, olefins and fatty acids, in peat samples subjected to heating at 350 °C for 0 and 180 s.

4. Conclusions

In general, the relative amounts of the major pyrolysis compounds follow progressive patterns in terms of temperature, which coincided with the behaviour observed by ^{13}C -NMR.

In the case of N-compounds, the positive correlation of the intensity of the ^{15}N NMR amide peak with the yield of indoles, imidazoles and pyrazoles, and the negative association with benzonitriles

and pyrazines could be used as a surrogate indicator of the extent of the heterocyclic N-domain in the peat under study.

It was observed that the most frequent N molecules after 120 s heating at 350 °C consisted of heterocyclic and aromatic products. At this stage, the biodiversity indices calculated from the yields of the N-compounds indicated increased complexity of the molecular assemblages with progressive heating. However, under more drastic conditions (in this experiment, after 180 s heating) both pyrolysis and NMR data, together with the calculated Shannon diversity indices, revealed an important reduction in both the relative abundance (number) and diversity of some groups of compounds, mainly methoxyphenols and N-containing compounds, such as pyrazine, methylpyrroline and pyrrole.

The quantitative and qualitative changes observed in the compound assemblages of the peat followed a two-stage transformation with progressive heating time. At relatively low heating intensities, the effect of the thermal treatment seemed to be mainly defunctionalisation, dealkylation and formation of new C and N structures, mainly at the expense of carbohydrate and peptides. This stage was followed by a steady state of selective concentration of the most stable constituents, mainly aromatic compounds but also polymethylene.

Both pyrolysis and ^{13}C -NMR data from the most drastically heated samples point to a resilient, condensed alkyl domain, together with a black-carbon-like condensed matrix in which *O*-alkyl constituents were almost completely depleted or transformed into aromatic structures.

Acknowledgements

The authors wish to thank Industries TOLSA, SA (Madrid) for providing the original peat and the Spanish CICYT for grant CGL-2008-04296. Dr. Ana Piedra Buena has been contracted by the CCMA-CSIC via the I3P Program, which is funded by the European Social Fund.

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