Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state ^{13}C- and ^{15}N-NMR spectroscopy

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Abstract

Peat samples subjected to thermal oxidation were studied by solid-state ^{13}C- and ^{15}N-NMR spectroscopy, so as to gain information on the thermal alteration of organic matter during processes such as natural fires or prescribed fires affecting soils. The ^{13}C-NMR spectra show that heating (up to 180 s at 350 °C) increases the aromaticity of the original peat to values typical for black-carbon material, but these charred residues (at least after weight losses of ca. 25%) still contained heat-resistant alkyl carbon. The ^{15}N-NMR spectrum of the original peat is dominated by a signal at −258 ppm, suggesting peptide-like material. In the heated samples a resonance line appearing at lower field indicates progressive formation of pyrrole- and indole-type compounds. Calculations based on weight loss, elemental composition and relative intensity distribution of the ^{13}C- and ^{15}N-NMR spectra demonstrate that the increasing concentrations of aromatic C-types and heterocyclic N-forms with progressive heating are not only caused by a relative enrichment concomitant with the selective thermal degradation of labile structures (mainly *O*-alkyl and amides) but correspond to newly-synthesised structures that were formed from aliphatic material.
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1. Introduction

From the different pools generally distinguished within soil organic matter (Balesdent and Mariotti, 1996) precise knowledge of the nature and fate of the most stable is important since variations in their abundance have a large influence on the CO_2 fluxes between the atmosphere and soils (Poirier et al., 2000). To this pool contribute the complex, polyaromatic substances collectively referred to as "black carbon" and generated by widespread burning processes.

Black carbon is widely distributed over the entire Earth's surface (Goldberg, 1985) and may account for a substantial portion of total organic C (up to 45%) in some soils (Haumaier and Zech, 1995; Skjemstad et al., 1996, 1997; Gustafsson and Gschwend, 1998; Glaser et al., 1998; Schmidt et al., 1999), mainly occurring as charred material produced by vegetation fires. There is also indirect evidence that such material may explain the origin of highly aromatic humic acids in volcanic ash soils (Hatcher et al., 1989; Golchin et al., 1997). Moreover, fire-induced reactions may substantially contribute to the terrestrial N sink (Knicker and Skjemstad, 2000). Research on the speciation patterns of N are of environmental interest, since more than half of the N in soil organic matter consists of unknown structures

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(Schnitzer, 1985). Recent NMR studies of the structure of organic N in soil and sedimentary organic matter have provided evidence for the dominance of amide forms in chiefly all types of sedimentary organic matter derived from microbial biomass or vascular plants (Almendros et al., 1991; Knicker et al., 1996a,b, 1997, 2000; Knicker and Hatcher, 1997). Thus, evidence for the classical assumption that the recalcitrant nitrogen-containing domain in humic substances largely consists of heterocyclic compounds has not been provided by NMR techniques.

It has been postulated that vegetation fires play a substantial role in the long term sequestration of C and N in forest soils (Parker et al., 2001). These processes should be understood better, since future significant changes in the C storage potential of soil may alter the global C cycle, leading to climatic uncertainties. However, systematic research on the effects of fires under environmental conditions is extremely complex due to the large number of processes involved in the composition of the disturbed soil samples (Almendros et al., 1984a, 1988, 1990). Simulation experiments based on laboratory-controlled burning of whole soil samples and isolated humic substances have yielded valuable information which can be more readily interpreted, although many uncertainties remain (Almendros et al., 1984b, 1992; Fernández et al., 1997). The main established changes in soil organic matter composition, as revealed by spectroscopic and wet chemical degradation methods, as well as laboratory incubation experiments, led to the conclusion that pyromorphic humus consists of rearranged macromolecular substances with reduced colloidal properties and increased resistance to biological degradation (Almendros et al., 1984a,b, 1988, 1990, 1992).

In order to elucidate the mechanisms involved in the alteration of organic materials subjected to thermal stress in soils and sediments, the present study deals with the effects of controlled heating on the quantitative distribution patterns of the different C and N forms in sapric peat [defined as having less than 33% fibre, i.e. plant residues retained on a 100 mesh (0.15 mm) sieve (Soil Survey Staff, 1996)] as assessed by solid state ^{13}C- and ^{15}N-NMR spectroscopy. The results are discussed in terms of stabilisation of the most refractory soil organic matter forms, which is of particular interest in studies of the global C and N biogeochemical cycles.

2. Experimental

2.1. Samples

The material used was collected from a sapric peat deposit located at Buyo (Galicia, Northern Spain). Details on the physico-chemical characteristics of this peat formation and its humic acid fraction have been published (Almendros et al., 1994).

The whole peat was homogenised to pass a 500 μm screen and subjected to dry heating according to experimental conditions described previously (Almendros et al., 1990, 1992). Briefly, samples of 1 g of peat were heated at 350 °C for variable periods. A series of triplicate samples in porcelain boats was introduced into a ceramic tubular electric furnace (Wosthöff o.H.G, Bochum) operating at atmospheric pressure and under a 625 ml min^{-1} suction flow. A membrane pump connected to a soda-lime column was used to supply CO_2-free air to the oxidation tube. After progressive oxidation times (60, 90, 120, 150 and 180 s) the boats were removed from the furnace and introduced into a desiccator with P_2O_5. This isothermal oxidation was shown to be more reproducible and easier to control than alternative heating for a constant time at different temperatures (Almendros et al., 1992).

The heated samples were weighed, corrections were made for the atmospheric moisture (independently calculated from samples heated at 100 °C overnight) and the elemental composition of the heated samples was determined using a Carlo Erba CHNS-O-EA1108 microanalyser and a ca. 7 mg sample.

2.2. NMR spectroscopy

The solid-state ^{13}C-NMR spectra were acquired with a Bruker DSX 200 spectrometer at a frequency of 50.32 MHz using a zirconium rotor of 7 mm outer diameter with KEL-F caps. The cross polarisation magic-angle spinning (CPMAS) technique (Schaefer and Stejskal, 1976) was applied with a frequency of 6.8 kHz. A ramped ^1H-pulse was used during contact time in order to circumvent spin modulation of Hartmann-Hahn conditions (Peersen et al., 1993; Cook et al., 1996). A contact time of 1 ms and a 90° ^1H-pulse width of 5.4 μs were used for all spectra. The ^{13}C-chemical shifts were calibrated relative to tetramethylsilane (0 ppm). Between 5000 and 8000 scans were accumulated using a pulse delay of 300 ms. The latter was recently shown to account for 5 T_{1H} of humic material, thus preventing saturation (Knicker et al., 1996a). Prior to Fourier transformation, a line broadening of 50 Hz was applied. Intensity distribution of the carbon atoms in different environments was determined by integration of signal intensity in various chemical shift regions using the instrument software. Owing to insufficient averaging of the chemical shift anisotropy at a spinning speed of 6.8 kHz, spinning side bands for the aromatic signals between 140 and 110 ppm occurred at a frequency distance of the spinning speed at both sides of the central signal. They are visible in the chemical shift region from 275 to 220 ppm and from 0 to −50 ppm and their intensity was added to that obtained for the 140–110

ppm region. Although the low field spinning side band of the peak in the region between 220 and 160 ppm is observable between 300 and 275 ppm, its high field spinning side band overlaps with the alkyl carbon region between 45 and 0 ppm. Consequently, for determination of the carbonyl carbon intensity, twice the low field spinning side band intensity was added to that of the region between 220 and 160 ppm. In the same way, 1-fold of this spinning side intensity was subtracted from the intensity in the region between 45 and 0 ppm to obtain the intensity of alkyl carbon.

Finally, the reliability of quantitative CPMAS data in solid-state NMR in the case of heterogeneous organic materials is a subject that has been discussed since NMR spectroscopy was introduced into studies of soils and geosciences. Due to the decrease in the H/C ratio of progressively heated materials there is a possibility of unequal efficiency in the cross-polarization which could make to some C atoms "invisible" if the C is more than 3 bonds apart than the nearest H (Alemany et al., 1983). Several authors have measured the optimal contact time at which the difference between the intensity loss of each chemical shift region is it a minimum. They have concluded that although not all C is seen, the relative intensity distribution is considered to be representative of the concentration distribution of the different chemical groups in the case of most types of humic substances (Fründ and Lüdemann, 1989). In particular, Freitas et al. (1999) failed to find significant differences between ^{13}C-NMR spectra obtained after direct excitation of ^{13}C and the cross-polarization spectra of peat samples heated up to 400 °C for 1 h; this led them to infer that the cross polarization spectra are in fact representative the overall carbon content of the material.

The solid-state CPMAS ^{15}N NMR spectra were obtained on a Bruker DMX 400 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. These parameters were recently optimised and shown to result in quantitative solid-state CPMAS ^{13}C NMR spectra of humic material (Knicker and Lüdemann, 1995). Between 5 and 7×10^5 scans were accumulated at a magic-angle spinning speed of 5.5 kHz. The chemical shifts were standardised to the nitromethane scale (0 ppm) and were adjusted using ^{15}N-labelled glycine (−347.6 ppm).

3. Results and discussion

3.1. Elemental composition

Figure 1 shows the weight loss of the peat material during progressive thermal oxidation. The kinetics follow a non-linear trend, since the least transformed sample studied (60 s) lost approximately 7% weight and the

sample after 180 s underwent a weight loss of approximately half of its original weight.

The changes in the elemental composition of the peat samples as a consequence of the progressive thermal oxidation are presented in Table 1. The data in Fig. 2 show that the loss of the major elements (C, H, O, N) also followed non-linear kinetics. The losses of H and O were the greatest. The charred residue tended to become enriched in those elements (C, N) present in the most resistant, or newly-formed, structures. These results coincided with the typical behaviour of soil humic and fulvic acids in the course of laboratory heating (Almendros et al., 1990), further confirmed and discussed in a more comprehensive paper (Baldock and Smernick, 2002). In fact, when the changes are plotted in a typical van Krevelen (1950) diagram (not shown), the progressive decrease in the H/C and O/C atomic ratios indicates the expected dehydration reactions in samples subjected to moderate heating (e.g., up to 90 s), whereas decarboxylation and demethylation became the dominant reactions in the longer-heated samples. The data in Table 1 are also relevant as regards the suitability of

Table 1
Elemental composition (ash-free, g·kg^{-1}) of peat subjected to progressive thermal oxidation as a function of oxidation time

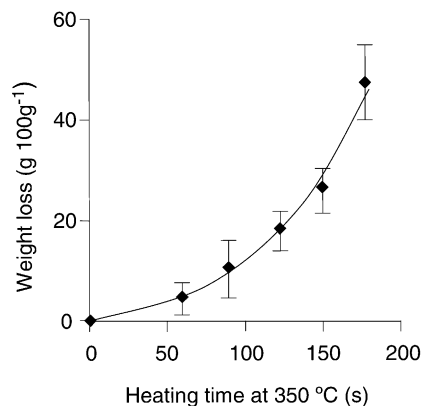| Oxidation time at 350 °C (s)	C	H	N	O[a]	Atomic ratios		C/N (w/w)
					H/C	O/C	
0	56.7	6.2	1.6	35.4	1.32	0.47	35.0
60	57.6	6.0	1.6	34.7	1.25	0.45	34.3
90	59.8	5.3	1.8	33.1	1.07	0.41	33.3
120	65.0	4.6	1.9	28.3	0.87	0.33	33.6
150	71.7	4.8	2.2	21.0	0.82	0.22	31.4
180	80.5	4.7	2.7	12.0	0.71	0.11	29.4

[a] Calculated by difference.

Fig. 1. Weight loss for peat samples subjected to thermal oxidation at 350 °C for variable periods between 60 and 180 s. Error bars indicate extreme values over triplicate experiments.

quantitative comparisons from the CPMAS spectral profiles. In fact, the degree of condensation of a sample can be estimated using the H/C ratio. Cross polarization becomes inefficient if the distance between C and H is larger than ca. 3 bonds. An H/C of 1.3 indicates that almost every C is bound to at least one H, namely all C should be visible using the CPMAS technique. The lowest H/C ratio is 0.7, indicating that one H is bound to 1.4 C. This is not low enough to assume large poly-aromatic structures in which the C is more than 3 bonds apart from the next H. Thus, for our samples it is not expected that in using CPMAS a considerable part of the aromatic C will be neglected.

Concerning the changes in the N content, it is clear that this element is not preferentially depleted, but has a tendency to be incorporated into structures fairly resis-
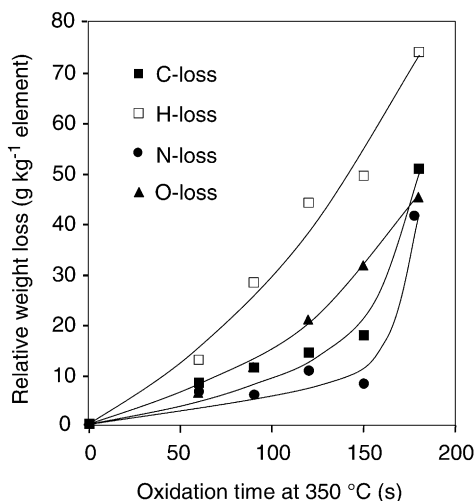

Fig. 2. Relative C, H, N, O losses during progressive thermal oxidation. The C, H and N-losses were calculated from the ash-free elemental analysis data and the determined weight loss (e.g., the N-loss (g kg^{-1} initial N) = 1000 × (1000 × (N × (1000-weight loss)/1000)/initial N). Similar calculations were performed for the other elements.

tant to heating. The C/N ratio remains relatively unchanged in samples subjected to moderate thermal oxidation (Table 1). In addition, the ratio decreases in the sample heated for more than 150 s, which seems to indicate the accumulation of recalcitrant organic N-forms in the charred material. A similar explanation was given concerning the decrease in the C/N ratio reported in soils affected by vegetation fires (Almendros et al., 1984a).

3.2. ^{13}C-NMR spectroscopy

Fig. 3 shows the solid-state CPMAS ^{13}C-NMR spectra of the original peat and the residues obtained after progressive heating. The relative percent distribution of the different C forms is given in Table 2.

The spectrum of the original peat shows, compared to spectra of fresh grass material (Knicker et al., 1996a), lower intensity in the chemical shift region between 110 and 60 ppm, commonly assigned to O-alkyl C atoms in carbohydrate-derived structures. The intense signal at 74 ppm and the resonance around 104 ppm are typical for C_2/C_3 and the anomeric (C_1) carbons in glycopyranosides, respectively.

Indications of the presence of lignin-derived structures are given by the signals in the chemical shift region of O-substituted aromatic C between 160 and 140 ppm. Those at 153 and 148 ppm can be attributed to C_3/C_5 in syringyl units and to C_3/C_4 in guaiacyl units, respectively (Lüdemann and Nimz, 1973). The signals for the remaining C in the aromatic rings contribute to the intense resonance line between 140 and 110 ppm. However, in this region some olefinic C may add to the intensity. The signal from the methoxyl C groups in lignin structures is expected to be around 56 ppm. Nevertheless, this chemical shift region overlaps with that of N-alkyl C. Considering the value of the atomic C/N ratio (ca. 41), and assuming that all of the N is bound to alkyl C, those C types should contribute <3% to the total intensity of this spectrum and thus could explain only half of the intensity found in the region between 60

Table 2
Intensity distribution in solid-state CPMAS ^{13}C-NMR spectra of sapric peat and residues obtained after progressive thermal oxidation

Oxidation time at 350 °C (s)	Carbonyl C 220–160 ppm	Aromatic C		O/N-Alkyl C			Alkyl C 45–0 ppm
		160–140 ppm	140–110 ppm	110–90 ppm	90–60 ppm	60–45 ppm	
0	11	8	19	7	19	6	30
60	12	9	21	6	16	6	31
90	13	11	23	5	12	5	32
120	11	14	29	6	7	5	28
150	12	16	37	5	2	4	25
180	12	19	49	5	1	2	13

and 45 ppm. The C of the propanyl side chains in lignin structures add, however, to the intensity in the chemical shift range between 90 and 60 ppm.

The ^{13}C-NMR region between 45 and 0 ppm is attributable to alkyl C, including dehydroxylated lignin side chains, amino acid residues, lipids and paraffinic structures. It is dominated by the signal peaking at 30 ppm typical of the internal methylene C in long alkyl chains. The small shoulder at the upfield site (14 ppm) originates from terminal methyl groups. Its low intensity relative to the signal at 30 ppm supports the presence of long-chain alkyl structures.

Peaks for carboxyl/amide and carbonyl C appear at 173 ppm and between 220 and 185 ppm, respectively. As revealed by the solid-state ^{15}N NMR spectrum of the original peat (Fig. 4), most of the nitrogen is bound in amide functional groups. The value of the C/N atomic ratio (41) of this sample indicates that only 2% of the total carbon occurs as amide-C. Some weak signals for spinning side bands for the aromatic structures are also detectable in the regions from 300 to 220 ppm and from 0 to −50 ppm.

Noticeable changes in the chemical composition of the peat were already detected in the samples subjected to moderate heating (up to 90 s). They are expressed in a minor relative enrichment of aromatic/olefinic C (160−110 ppm) and a declining contribution of O/N-alkyl C (110–45 ppm) which decreased from 32% of the C in the untreated peat to 22% in the sample heated for 90 s (Table 2). The relative intensities in the chemical shift regions of carbonyl C (220–160 ppm) and alkyl C (45–0 ppm) remain fairly constant.

Further alteration of the peat chemical composition in terms of heating are featured by a continuous increase of the relative intensity in the sp^2-C region (160–110 ppm) occurring mainly at the expense of the intensity in the O/N-alkyl region. The progressive heating led to an increase in the aromaticity of the original peat to values typically found for charred material (Almendros et al., 1992). This can be explained by two phenomena: the relative enrichment of lignin residues

that are reported to exhibit a higher resistance towards thermal treatment than O/N alkyl and an intense charring of carbohydrate moieties (Almendros et al., 1990; Pastorova et al., 1994; Knicker et al., 1996a; Baldock and Smernik, 2002). This suggestion is supported by the observation that in the ^{13}C NMR spectrum of the sample heated for 120 s, the signals in

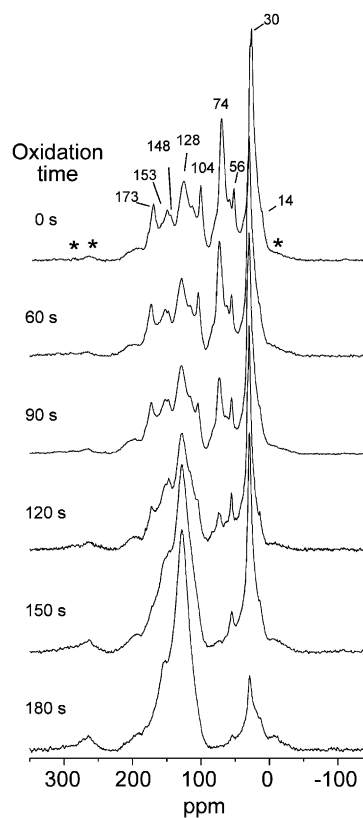Fig. 3. Solid-state CPMAS ^{13}C-NMR spectra of peat samples subjected to progressive thermal oxidation at 350 °C for different periods up to 180 s. Asterisks indicate spinning side bands.

Table 3
Recovery of each C type during progressive thermal oxidation, calculated from relative intensity distribution in Table 2 and calculated total C-loss (Table 1)

Oxidation time at 350 °C (s)	Carbonyl C 220–160 ppm	Aromatic C			O/N-Alkyl C				Alkyl C 45–0 ppm
		160–140 ppm	140–110 ppm	Total	110–90 ppm	90–60 ppm	60–45 ppm	Total	
0	100	100	100	100	100	100	100	100	100
60	97	101	100	100	81	79	85	81	96
90	107	116	108	111	66	54	76	61	94
120	89	141	134	136	73	32	76	49	80
150	87	163	162	162	59	10	47	28	68
180	52	113	128	124	37	3	14	12	22

the chemical shift region of *O*-aryl C (160 to 140 ppm) and methoxyl C (60–45 ppm) are still visible, while that in the region of anomeric C (110–90 ppm) became indiscernible from the broad signal peaking at 128 ppm. For this spectrum, the intensity between 90 and 60 ppm declined. With increasing treatment time, the carbohydrate-related signals vanish completely whereas the alkyl C intensity, which was only slightly affected up to 120 s, exhibits a considerable decrease at the end of the experiment. Here, however, it has to be noted that the intensity in the methoxyl C region continuously declines with ongoing heat treatment. This suggests that the lignin residues are selectively enriched in spite of the loss of their methoxyl functionality.

Apart from this selective enrichment in structures inherited from resistant plant macromolecules, a neosynthesis of unsaturated compounds [formation of furan compounds is classically referred to (Baldock and Smernik, 2002)] should have occurred. According to the significant loss of H (Fig. 2), this may result from
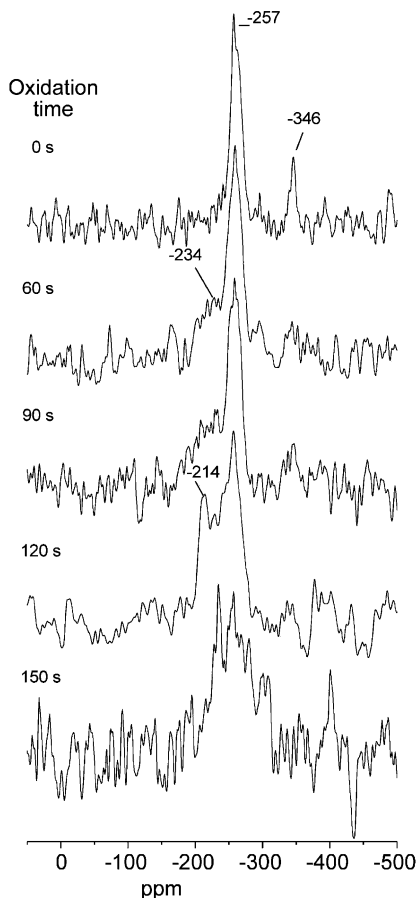

Fig. 4. Solid-state CPMAS ^{15}N-NMR spectra of peat samples subjected to progressive thermal oxidation at 350 °C for different periods up to 150 s. The chemical shift scale is reference to nitromethane (0 ppm).

carbohydrate dehydration and transformations of alkyl structures concomitant with the formation of double bonds. In fact, in the case of the heavily heated samples, it is to be expected that most of the C in the 160–110 ppm region would correspond to aromatic structures originating from charred carbohydrate, as suggested by Pastorova et al., (1994) and Almendros et al. (1997).

The synthesis indicated above and probable cyclisation of unsaturated structures affect not only the intensity of the chemical shift region between 140 and 110 ppm but also that between 160 and 140 ppm. In fact, with progressive heating, the ratio between their intensities does not change considerably (Table 2). This would indicate the formation and accumulation of *O*-aryl or C-substituted unsaturated structures.

During prolonged heat treatment, approximately 50% of the original carboxyl/carbonyl/amide C (maximum at 173 ppm) was lost (Table 3). Most of the remaining intensity is overlapped by the broad aromatic signal, which may indicate a shift towards higher field. This would point to carboxyl C connected to aromatic rings (Fig. 3).

In contrast to previous studies (Knicker et al., 1996a) of the thermally induced changes in N-rich, fresh grass material, where most of the intensity in this region was attributable to amides, in the spectrum of the original peat, amide carbon could only account for 20% of the total intensity. Thus, heat-induced degradation of peptide structures had a greater influence on the carboxyl/carbonyl/amide C in grass material than in the peat studied here.

The charred residue, corresponding to the most transformed material, chiefly consists of aromatic and alkyl C, a ^{13}C-NMR profile (Fig. 3) that has often been compared to that of mature kerogens (Wilson, 1987). The region between 45 and 0 ppm shows a shoulder at ca. 20 ppm that could be assigned to methyl groups linked to aromatic moieties. Considering these methyl groups, in addition to those occurring at 14 ppm, it appears that the contribution of polymethylene structures to this area is comparatively low. In fact, the final residue (180 s), that still contains an appreciable amount of alkyl C (Table 2), has been suggested to consist not only of long-chain residual paraffinic structures from e.g., plant waxes, but also of some kind of methyl, methylene or alicyclic structures that may be "fixed" as recalcitrant C forms in predominantly aromatic matrices (González-Vila et al., 2001). In particular, the existence of a similar alkyl component in pseudo-melanoidins (N-lacking, sugar-derived, abiotically-synthesised macromolecules) which should not be susceptible to chemical or biological degradation (Almendros et al., 1997) could be invoked to explain such heat-resistant aliphatic structures in black carbon-type materials.

As discerned from Table 3 where the relative intensities of the C-signals are related to the calculated total C-loss

for the different C-types during progressive heating, the decrease in *O*-alkyl C can explain most of the C-loss. Some *O*-alkyl C, however, may have already been chemically transformed into e.g., furans, which could explain the increase in the amounts of *O*-aromatic C. The data in Table 3, where values greater than 100 indicate thermal neoformation reactions for the corresponding structures, are especially useful for differentiating selective depletion from accumulation of newly-formed C forms. Thus, a net increase (up to 62%) for aromatic carbons after a heating time of 150 s was observed. On the other hand, the negative balance for the aliphatic C-forms was evident in the *O*-alkyl structures, where only 12% remains at the end of the experiment.

The above results point to the existence of successive stages during the thermal alteration, similar to those indicated by previous studies with different organic materials (Almendros et al., 1982, 1999; González-Vila et al., 2001). The early stages would correspond to thermal diagenetic transformations affecting mainly oxygen-containing groups, where dehydration and decarboxylation would turn pyranoside structures into unsaturated rings. In these stages, presumably leading to shrinking and internal cross-linking of originally flexible, "open" macromolecular three-dimensional structures, molecular encapsulation of aliphatic molecules could occur. In the advanced stages, the accumulation of polycyclic benzene rings and the removal of less stable alkyl moieties accumulated in the rigid matrix of the charred material could occur. The latter processes, coinciding with a decrease in the H/C atomic ratio, could be assimilated into a "thermal distillation" of hydrocarbons and other compounds of reduced reactivity not suitable for forming covalent bonds through endothermic reactions (González-Vila et al., 2001).

3.3. ^{15}N-NMR spectroscopy

The CPMAS ^{15}N-NMR spectra of the original and the progressively heated peat samples are shown in Fig. 4. The relative intensity distribution of the different N forms is given in Table 4. The spectra are dominated by prominent signals ranging from −220 to −285 ppm, with a maximum around −259 ppm. In this region, resonances from amides, peptides, indoles, lactams and carbazoles are expected (Witanowski et al., 1993). Similar spectral features were reported for compost (Pichler et al., 2000), soils and sediments (Knicker et al., 1996b, 2000; Knicker and Hatcher, 1997; Knicker, 2000; Knicker and Skjemstad, 2000). This was generally interpreted as a dominance of amide N in peptides. The respective signal from their free amino groups is observed at −346 ppm. Based on chemical and thermal degradation procedures, N-containing heterocyclic aromatics such as indoles, pyrroles or pyridines have traditionally been postulated to be structural units of soil humic fractions (Schulten and Schnitzer, 1998). These compounds give signals in the lower field range of the spectrum between −145 and −250 ppm (−25 and −145 for pyridine-type compounds and between −145 and −240 for pyrrole-type structures). Thus, the chemical shift region of indoles (around −245 ppm) slightly overlaps the amide region, although if such compounds were major components of the organic N in the untreated peat, the broad signal would be shifted toward lower field. In addition, in the spectrum of the original peat the chemical shift region between −145 and −250 ppm accounts for only 9% of the total intensity, indicating that such structures do not occur to a great extent (Table 4).

From the ^{15}N-NMR spectra, it appears that heating in air leads to a continuous increase in the relative intensity of the chemical shift region for heterocyclic N, which is especially noted in the sample heated for 120 s (Table 4). Already, heat application for 60 s induces changes in the composition of the N structures, indicated by the relative increase in the region of heterocyclic N, at the expense of that assigned to amide N. This tendency continues up to a heating time of 120 s. However, around 40% of the total intensity of the spectrum is still attributable to amide N structures (Table 4), indicating a higher resistance of these compounds

Table 4
Intensity distribution in solid-state CPMAS ^{15}N NMR spectra of sapric peat and residues obtained after progressive thermal oxidation

Oxidation time at 350 °C (s)	Pyridine-type N −25–(−145) ppm	Pyrrole-type N −145–(−240) ppm	Amide N −240–(−285) ppm	Amino N	
				−285–(−300) ppm	−330–(−350) ppm
0	1	8	75	4	12
60	2	31	58	4	5
90	1	41	51	0	7
120	9	47	39	1	3
150	7	43	43	7	0

towards thermal degradation, at least compared with *O*-alkyl and carbonyl groups that, at this stage, have been to a large extent transformed or removed. The above persistence of amide structures may be explained by stabilizing cross-linking reactions, including the formation of Maillard products (sugar-amino acid reactions; Ellis, 1959), which has been suggested to occur during peat formation (Nguyen and Harvey, 1998). Another possibility would be steric hindrance in e.g., organic domains from which both the transfer of energy and the ready volatilisation of thermal degradation fragments would be comparatively impeded with respect to structures in the most external zones (Knicker and Skjemstad, 2000). At this point, we should also take into account the possibility that the resistance of some structural moieties, e.g. alkyl, could to some extent depend on the non-homogeneous composition of particulate material after heating. In spite of the low particle size of the material under study, charring often leads to materials that, in the intermediate stages, could consist of particles where the core is comparatively less transformed than the most external regions. Nevertheless, the high resistance to acid hydrolysis of the C and N forms in the charred fractions suggests that the influence of this effect is not the main source of heterogeneity in particulate organic matter (Almendros et al., 1984a,b).

The relative enrichment of heterocyclic aromatic N during thermal treatment, occurring concomitantly with the decrease in amide N, was also reported from pyrolysis studies of algaenan (Derenne et al., 1993) and of grass material (Knicker et al., 1996a). At first sight, this fact might be interpreted as resulting from the selective preservation of heteroaromatic structures that were already present in low amounts in the untreated peat, while the more labile peptide structures are preferentially thermally degraded. Nevertheless, when the relative intensity distribution in the ^{15}N NMR spectra is calculated with regard to N-loss, another scenario becomes evident. As shown in Fig. 5, after 60 s of heat treatment, the percentage of the N bound in pyrroles or indoles increased about four times from the untreated sample to that heated for 120 s. This gives clear evidence that those compounds were newly formed, possibly by auto-condensation reactions from NH_3 released from amide-containing moieties and aromatic compounds, including those occurring in the advanced carbohydrate dehydration and Maillard-type reactions. Further reactions that may contribute to the formation of heterocyclic N structures involve cyclisation of aliphatic chains in the presence of amino groups or NH_3, or the cyclisation of peptide chains. The formation of such heterocyclic compounds would also add to the increase in relative intensity in the region between 140 and 110 ppm in the solid-state ^{13}C-NMR spectra.

Until 90 s heating, the amount of accumulated pyrrole- and indole-type structures shows no considerable
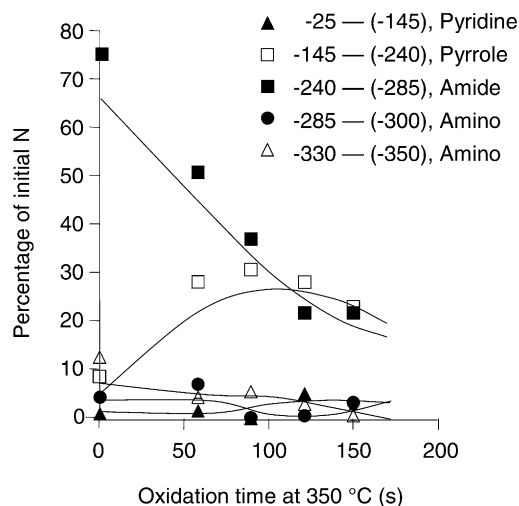

Fig. 5. Distribution of N-compounds in the different chemical shift regions as relative intensities of the ^{15}N signals (Table 4) calculated from N-losses during progressive thermal oxidation.

increase with respect to the untreated sample and the sample heated for 60 s. After a treatment time of 120 s a slight decrease is observable. However, due to the low N content of this sample, only a weak signal-to-noise ratio was achieved, obscuring phase and baseline correction and thus integration. Hence, this decrease may be due to the poor quality of this spectrum. In particular, Benzing-Purdie et al. (1983) showed the presence of pyrrole and indole N-forms in the ^{15}N NMR spectra of melanoidins from xylose and ammonium sulfate obtained by heating 1M solutions at 68 °C for 30 days. Besides heterocyclic N structures, melanoidins are known to include amide bonds (Benzing-Purdie et al., 1983), which may support the explanation for the accumulation of resistant amide functional groups after severe heating.

4. Conclusions

The solid-state ^{13}C-NMR spectra suggest two partially overlapping processes during thermal transformation of the peat organic matter. Under relatively mild heating conditions the main processes are dehydration and selective degradation of *O*-alkyl structures; the breakdown of macromolecular structures releases reactive compounds of low molecular weight. During successive stages, the endothermic condensation reactions of these products lead to macromolecular substances including newly-formed aromatic constituents from which more stable alkyl molecules are progressively removed by thermal distillation. Only a portion of recalcitrant, cyclic or branched paraffinic structures remain in a condensed unsaturated matrix. The weight loss values in addition to the ^{13}C and ^{15}N

signal intensities indicate that both aromatic and heterocyclic N-containing structures which did not exist in the original peat are formed as a result of heating. Nevertheless, it also appeared that most of this recalcitrant, "unknown" N-forms still consists of amide structures remaining in black carbon-like soil organic matter that have been found to be relatively stable against chemical, biological and thermal degradation (Almendros et al., 1990).

In conclusion, the results indicate that heating peat organic matter involves aromatisation and neoformation of heterocyclic N. From the biogeochemical viewpoint these can be considered as mechanisms leading to the transformation of labile compounds into environmentally recalcitrant forms.

Associate Editor—**Claude Largeau**

References

Alemany, L.B., Grant, D.M., Pugmire, R.J., Alger, T.D., Zilm, K.W., 1983. Cross polarization and magic angle sample spinning NMR spectra of model organic compounds. 2. Molecules of low or remote protonation. Journal of the American Chemical Society 105, 2142–2147.

Almendros, G., Polo, A., Vizcayno, M.C., 1982. Application of thermal analysis to the study of several Spanish peats. Journal of Thermal Analysis 24, 175–182.

Almendros, G., Polo, A., Ibáñez, J., Lobo, M.C., 1984a. Contribución al estudio de la influencia de los incendios forestales en las características de la materia orgánica del suelo. I: transformaciones del humus en un bosque de *Pinus pinea* del Centro de España. Revue d'Ecologie et de Biologie du Sol 21, 7–20.

Almendros, G., Polo, A., Lobo, M.C., Ibáñez, J., 1984b. Contribución al estudio de la influencia de los incendios forestales en las características de la materia orgánica del suelo. II: transformaciones del humus por ignición en condiciones controladas de laboratorio. Revue d'Ecologie et de Biologie du Sol 21, 145–160.

Almendros, G., Martin, F.J., González-Vila, F.J., 1988. Effects of fire on humic and lipid fractions in a Dystric Xerochrept in Spain. Geoderma 42, 115–127.

Almendros, G., González-Vila, F.J., Martin, F., 1990. Fire-induced transformation of soil organic matter from an oak forest. An experimental approach to the effects of fire on humic substances. Soil Science 149, 158–168.

Almendros, G., Fründ, R., González-Vila, F.J., Haider, K.M., Knicker, H., Lüdemann, H.-D., 1991. Analysis of ^{13}C and ^{15}N CPMAS NMR-spectra of soil organic matter and composts. FEBS Letters 282, 119–121.

Almendros, G., González-Vila, F.J., Martin, F., Fründ, R., Lüdemann, H.-D., 1992. Solid state NMR studies of fire-induced changes in the structure of humic substances. Science of the Total Environment 117/118, 63–74.

Almendros, G., Fründ, R., Martin, F., González-Vila, F.J., 1994. Spectroscopic characteristics of derivatized humic acids from peat in relation to soil properties and plant growth. In: Senesi, N., Miano, T.M. (Eds.), Humic Substances in the Global Environment and Implications in Human Health. Elsevier Science, BV, pp. 213–218.

Almendros, G., Dorado, J., González-Vila, F.J., Martin, F., 1997. Pyrolysis of carbohydrate-derived macromolecules: its potential in monitoring the carbohydrate signature of geopolymers. Journal of Analytical and Applied Pyrolysis 40-41, 599–610.

Almendros, G., . Dorado, J., González-Vila, F.J., Blanco, M.J., Lankes, U., 1999. ^{13}C NMR assessment of decomposition patterns during composting of forest and shrub biomass. Soil Biology and Biochemistry 32, 793–804.

Baldock, J.A., Smernik, R.J., 2002. Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. Organic Geochemistry 33, 1090–1109.

Balesdent, J., Mariotti, A., 1996. Measurement of soil organic matter turnover using ^{13}C natural abundance. In: Boutton, T.W., Yamasaki, S.I. (Eds.), Mass Spectrometry of Soils. Dekker, New York, pp. 83–111.

Benzing-Purdie, L., Ripmeester, J.A., Preston, C.M., 1983. Elucidation of the nitrogen forms in melanoidins and humic acid by nitrogen-15 cross polarization-magic angle spinning nuclear magnetic resonance. Journal of Agriculture and Food Chemistry 31, 913–915.

Cook, R.L., Langford, C.H., Yamdagni, R., Preston, C.M., 1996. A modified cross-polarization magic angle spinning ^{13}C NMR procedure for the study of humic materials. Analytical Chemistry 68, 3979–3986.

Derenne, S., Largeau, C., Taulelle, F., 1993. Occurrence of non-hydrolysable amides in the macromolecular constituent of *Scenedesmus quadricauda* cell wall as revealed by ^{15}N NMR: Origin of *n*-alkylnitriles in pyrolysates of ultra-laminae-containing kerogens. Geochimica et Cosmochimica Acta 57, 581–587.

Ellis, G.P., 1959. The Maillard reaction. Advances in Carbohydrate Chemistry 14, 63–134.

Fernández, I., Cabaneiro, A., Carballas, T., 1997. Organic matter changes immediately after a wildfire in an Atlantic forest soil and comparison with laboratory soil heating. Soil Biology and Biochemistry 29, 1–11.

Freitas, J.C.C., Bonagamba, T.J., Emmerich, F., 1999. ^{13}C High-resolution solid-state NMR study of peat carbonisation. Energy and Fuels 13, 53–59.

Fründ, R., Lüdemann, H.D., 1989. The quantitative analysis of solution- and CPMAS-C-13 NMR spectra of humic material. The Science of the Total Environment 81/82, 157–168.

Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 1998.

Black carbon in soils: the use of benzenecarboxylic acids as specific markers. Organic Geochemistry 29, 811–819.

Golchin, A., Clarke, P., Baldock, J.A., Higashi, T., Skjemstad, J.O., Oades, J.M., 1997. The effects of vegetation and burning on the chemical composition of soil organic matter in a volcanic ash soil as shown by ^{13}C NMR spectroscopy. I. Whole soil and humic acid fraction. Geoderma 76, 155–174.

Goldberg, E.D., 1985. Black Carbon in the Environment. Wiley, New York.

González-Vila, F.J., Tinoco, P., Almendros, G., Martin, F., 2001. Py-GC-MS analysis of the formation and degradation stages of charred residues from lignocellulose biomass. Journal of Agricultural and Food Chemistry 49, 1128–1131.

Gustafsson, O., Gschwend, P.M., 1998. The flux of black carbon to surface sediments on the New England continental shelf. Geochimica et Cosmochimica Acta 62, 465–472.

Hatcher, P.G., Schnitzer, M., Vasallo, A.M., Wilson, M.A., 1989. The chemical structure of highly aromatic humic acids in three volcanic ash soils as determined by dipolar dephasing NMR studies. Geochimica et Cosmochimica Acta 53, 125–130.

Haumaier, L., Zech, W., 1995. Black carbon—possible source of highly aromatic components of soil humic acids. Organic Geochemistry 23, 191–196.

Knicker, H., Lüdemann, H.-D., 1995. N-15 and C-13 CPMAS and solution NMR studies of N-15 enriched plant material during 600 days of microbial degradation. Organic Geochemistry 23, 329–341.

Knicker, H., Almendros, G., González-Vila, F.J., Martin, F., Lüdemann, H.-D., 1996a. ^{13}C- and ^{15}N-NMR spectroscopic examination of the transformation of organic nitrogen in plant biomass during thermal treatment. Soil Biology and Biochemistry 28, 1053–1060.

Knicker, H., Scaroni, A.W., Hatcher, P., 1997. ^{13}C- and ^{15}N-NMR spectroscopic investigation on the formation of fossil algal residues. Organic Geochemistry 24, 661–669.

Knicker, H., Lüdemann, H.-D., Haider, K., 1997. Incorporation studies of NH_4^+ during incubation of organic residues by ^{15}N-CPMAS-NMR-spectroscopy. European Journal of Soil Science 48, 431–441.

Knicker, H., Hatcher, P.G., 1997. Survival of protein in organic-rich sediments. Possible protection by encapsulation in organic matter. Naturwissenschaften 84, 231–234.

Knicker, H., Schmidt, M.W.I., Kögel-Knabner, I., 2000. Immobilization of peptides in fine particle size separates of soils as revealed by NMR spectroscopy. Soil Biology and Biochemistry 32, 241–252.

Knicker, H., Skjemstad, J.O., 2000. Carbon and nitrogen functionality in protected organic matter of some Australian soils as revealed by solid-state ^{13}C- and ^{15}N-NMR. Australian Journal of Soil Science 38, 113–127.

Knicker, H., 2000. Biogenic nitrogen in soils as revealed by solid-state ^{13}C and ^{15}N NMR spectroscopy. Journal of Environmental Quality 29, 715–723.

Krevelen van, D.W., 1950. Graphical-statistical method for the study of structure and reaction processes of coal. Fuel 29, 269–284.

Lüdemann, H.-D., Nimz, H., 1973. Carbon-13 nuclear magnetic resonance spectra of lignins. Biochemical and Biophysical Research Communications 52, 1162–1169.

Nguyen, R.T., Harvey, H.R., 1998. In: Stankiewicz, B.A., Van Bergen, P.F. (Eds.), Nitrogen-Containing Macromolecules in the Bio- and Geosphere. ACS Symposium Series 707; American Chemical Society, Washington, DC, pp 88–112.

Parker, J.L., Fernández, I.J., Rustad, L.E., Norton, S.A., 2001. Effects of nitrogen enrichment, wildfire, and harvesting on forest soil carbon and nitrogen. Soil Science Society of America Journal 65, 1248–1255.

Pastorova, I., Botto, R.E., Arisz, P.W., Boon, J.J., 1994. Cellulose char structure: a combined analytical Py-GC-MS, FTIR, and NMR study. Carbohydrate Research 262, 27–47.

Peersen, O.B., Wu, X., Kustanovich, I., Smith, S.O.J., 1993. Variable-amplitude cross-polarization MAS NMR. Journal of Magnetic Resonance 104, 334–339.

Pichler, M., Knicker, H., Kögel-Knabner, I., 2000. Changes in the chemical structure of municipal solid waste during composting as studied by solid-state dipolar dephasing and PSRE ^{13}C NMR and solid-state ^{15}N NMR spectroscopy. Environmental Science and Technology 34, 4034–4038.

Poirier, N., Derenne, S., Rouzaud, J.-N., Largeau, C., Mariotti, A., Balesdent, J., Maquet, J., 2000. Chemical structure and sources of the macromolecular, resistant, organic fraction isolated from a forest soil (Lacadee, South-west France). Organic Geochemistry 31, 813–827.

Schaefer, J., Stejskal, E.O., 1976. Carbon-13 nuclear magnetic resonance of polymers spinning at magic angle. Journal of the American Chemical Society 98, 1031–1032.

Schmidt, M.W.I., Skjemstad, J.O., Gehrt, E., Kögel-Knabner, I., 1999. Charred organic carbon in German chernozemic soils. European Journal of Soil Science 50, 351–365.

Schnitzer, M., 1985. Nature of nitrogen in humic substances. In: Aiken, R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (Eds.), Humic Substances in Soil Sediment and Water. Wiley-Interscience, New York, pp. 303–325.

Schulten, H.-R., Schnitzer, M., 1998. The chemistry of soil organic nitrogen: a review. Biology and Fertility of Soils 26, 1–15.

Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., McClure, S.G., 1996. The chemistry and nature of protected carbon in soil. Australian Journal of Soil Research 34, 251–271.

Skjemstad, J.O., Clarke, P., Golchin, A., Oades, H.M., 1997. Characterization of soil organic matter by solid state ^{13}C NMR spectroscopy. In: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford, pp. 253–271.

Soil Survey Staff, 1996. Keys to Soil Taxonomy. USDA Natural Resource Conservation Service, seventh ed. US Government Printing Office, Washington DC.

Wilson, M.A., 1987. NMR Techniques and Applications in Geochemistry and Soil Chemistry. Pergamon Press, Oxford, UK.

Witanowski, M., Stefaniak, L., Webb, G., 1993. Nitrogen NMR spectroscopy. In: Webb, G.A. (Ed.), Annual Reports on NMR Spectroscopy 25. Academic Press, London.