A solid-state ^{15}N NMR spectroscopic investigation of the origin of nitrogen structures in coal

H. Knicker ^{a,*}, P.G. Hatcher ^{a,b}, A.W. Scaroni ^{a,b}

^a *Energy and Fuels Research Center, 209 Academic Projects Building, The Pennsylvania State University, University Park, PA 16802, USA*

^b *Fuel Science Program, 209 Academic Projects Building, The Pennsylvania State University, University Park, PA 16802, USA*

Solid-state ^{15}N nuclear magnetic resonance (NMR) spectroscopy is a technique that has been available for the study of nitrogen compounds for many years but only recently has been applied to the study of nitrogen in coal due to the extremely low sensitivity of the ^{15}N -isotope. Reported here is the application of ^{15}N NMR to a maturation series of undegraded plant material, plant composts, sediments, and coal samples for the purpose of investigating the alterations of nitrogen functional groups during peat and coal formation. Spectroscopic parameters such as relaxation times and cross polarization dynamics were determined for ^{15}N -enriched plant composts to establish the optimal NMR conditions to be used for natural abundance ^{15}N measurements on samples not enriched in ^{15}N .

The results show that up to the peat stage most of the nitrogen occurs as amide nitrogen, which derives from biogenic precursors (presumably proteins). Upon increasing coalification, pyrrolic-N becomes the dominant form in the macromolecular coal network. Pyrrolic-N in coals may be derived from selective preservation of biogenic pyrroles or by rearrangement of amide chains during maturation. Pyridinic-N does not appear to be a major constituent of coal nitrogen of the coals studied here. Our successful application of solid-state ^{15}N NMR to coal samples has provided new insights and understanding of coal-nitrogen chemistry.

Keywords: ^{15}N -NMR; nitrogen in coal; sediment diagenesis; selective preservation

1. Introduction

Coal is a macromolecular organic material, derived from the burial and compaction of peat formed under various wetland conditions (Stach et al., 1982; Hatcher et al., 1983; Teichmüller, 1989; Mukhopadhyay and Hatcher, 1993). It is composed mainly of organic matter with various amounts of inorganic compounds. The main atomic constituents are carbon, oxygen and hydrogen. Nitrogen and sulfur are minor organic components and usually occur in concentrations of less than 2% by weight (Glick and Davis, 1991).

The nitrogen in the macromolecular organic network of coal most likely originates from plant debris, microbial residues and algal remains. Nitrogen in these biogenic materials exists mainly as amide and amino functional groups in proteinaceous material. During early diagenesis in the predominantly anaerobic environment in peats, portions of these remains are microbiologically mineralized, while other portions are protected from microbial degradation. The residual organic nitrogen in peat is preserved and accumulates to eventually form coal (Mukhopadhyay and Hatcher, 1993). Coalification caused by prolonged burial and increasing temperature and pressure leads to physico-chemical transformations of the preserved nitrogenous biopolymers.

In general, there are two theories that explain the alteration of nitrogenous biomolecules into complex nitrogen-containing macromolecules existing in sedimentary rocks. The first theory invokes the condensation-polymerization model whereby biomaterial is degraded first into small molecules (amino acids, phenols and carbohydrates) and then these recombine into a macromolecular structure by abiotic condensation processes. The nitrogen from the amino acids is believed to be incorporated into heterocyclic compounds and aniline derivatives both formed via autopolymerization reactions with lignin degradation products (Flaig et al., 1975) or by the Maillard reaction of sugars with amino compounds (Maillard, 1917; Anderson et al., 1989). These reactions presumably would occur during early peat formation and should be recognizable as an enrichment of the heterocyclic products with increasing peat maturation. Benzing-Purdie et al. (1983) have shown that such reactions are theoretically possible; however, significant amounts of the nitrogen-containing products expected from these reactions could not be identified in soils and sediments (Schnitzer, 1985).

The second theory regarding maturation of organic material in sediments is based upon the selective preservation model (Hatcher et al., 1983; Tegelaar et al., 1989). This model describes maturation as a thermal alteration of only the most resistant biopolymers surviving the early stages of microbial degradation. These resistant biopolymers are transformed further to varying degrees to form the backbone structure of the coal macromolecules. Hatcher et al. (1989a,b) examined the coalification of xylem from gymnosperm and angiosperm wood and clearly showed that vitrinitic coal structure evolves from lignin polymers. The non-lignin components of vascular, as well as nonvascular plants, are also thought to be subjected to a selective preservation process, whereby the most resistant components eventually become the dominant contributors to the surviving coal. A comparison of the microlaminae in algal deposits by transmission electron microscopy with those of modern algae revealed a high physical similarity (Largeau et al., 1984, 1986, 1990). Apparently the refractory biopolymers of algae,

including those containing nitrogen compounds, are concentrated and selectively preserved in these microlaminae (Tegelaar et al., 1989).

The chemical nature of nitrogen in coal has long been of interest for a variety of reasons. First, nitrogen-containing structures in coal are well known to be involved in the release of nitrogen oxides during coal combustion (Aho et al., 1993). Second, many nitrogen-containing polycyclic aromatic compounds in coal and coal-derived liquids are mutagenic and/or carcinogenic (Lee et al., 1981). Third, nitrogen functional groups have a major effect on fuel stability, refinery catalysts and the solubility of coal derived liquids (Snape and Bartle, 1985). Finally, nitrogen compounds are believed to play an important role in linking together the macromolecular subunits of the coal structure (Shinn, 1984). Although nitrogen in coal is believed to occur principally in pyridine and pyrrole analogs, the origin and chemistry of the nitrogen-containing structures during coalification are not well understood (Attar and Hendrickson, 1982).

Principally, there are two approaches for the investigation of the chemical structure of nitrogen in coal. The first involves thermolytic or chemolytic degradation of the macromolecule into small fragments that are analyzed by gas chromatography or gas chromatography-mass spectrometry. It is assumed that the fragments are representative of the original larger coal molecules. Because secondary reactions (rearrangement, cracking, hydrogenation and polymerization) cannot be excluded, it is obvious that conclusions regarding the original structure of nitrogen in the macromolecular phase have to be drawn with caution.

Alternative techniques for the examination of nitrogen in heterogeneous macromolecular mixtures are nondestructive spectroscopic methods, which include solid-state ^{15}N nuclear magnetic resonance (NMR) spectroscopy (Knicker et al., 1995a), X-ray absorption edge spectroscopy (Kirtley et al., 1993), and X-ray photoelectron spectroscopy (Burchill and Welch, 1989; Wójtowicz et al., 1995). Although solid-state ^{13}C NMR is a rather well established technique for the analysis of carbon structures (Wilson, 1987), the development of solid-state ^{15}N NMR of nitrogen-containing structures has been hampered by two factors. First, the most abundant nitrogen isotope ^{14}N has a large quadrupole moment from which it is impossible to obtain high resolution ^{14}N NMR spectra (Witanowski et al., 1993). Second, the dipolar ^{15}N nucleus, unfortunately, has a low natural abundance of 0.37% and a low and negative gyromagnetic ratio. Therefore, the sensitivity of ^{15}N NMR is approximately 50 times lower than ^{13}C NMR. Consequently, ^{15}N NMR has not been used widely for studies of coal and related geopolymers, other than the studies involving ^{15}N -enriched material (Ripmester et al., 1986; Cheshire et al., 1990; Derenne et al., 1993). Until now, natural abundance ^{15}N NMR on coal and related materials has been virtually impossible. The recently successful application of ^{15}N NMR to the study of organic matter in soil systems at natural ^{15}N abundance levels (Knicker et al., 1993, 1996) suggests that solid-state ^{15}N NMR spectra can be used to study the structure of nitrogen in the macromolecular network of coals at natural abundance ^{15}N -levels (Knicker et al., 1995a).

To obtain suitable ^{15}N NMR spectra, it was necessary to determine the proper experimental conditions which provided acceptable signal-to-noise ratios from ^{15}N -enriched plant composts. Enriched composts provided a strong signal for the optimization of instrumental parameters. The enriched composts were also incubated under laboratory

conditions simulating wetland conditions present during early peat formation. Once the optimized parameters were determined, they were used to obtain natural abundance ^{15}N NMR spectra of various sediments (peats) and coal samples. The methodologies and data obtained are presented as they relate to the ^{15}N NMR studies of coal and related materials. Our focus was on the coalification or maturation process and how it affects the nature and distribution of nitrogen-containing compounds. Thus, the samples examined included peats and coals representing a maturation series. We also sought to focus on modern and ancient analogs of both humic and sapropelic coals.

2. Materials and methods

2.1. Sample preparation and origins

2.1.1. Undegraded material and composts

Lolium perenne was grown on a nutrient solution containing potassium nitrate (approx. 99.7 atom% ^{15}N) (Hewitt, 1966) as the sole nitrogen source. Plant material was collected at 14-day intervals, freeze-dried, and then milled into a fine powder. Wheat (*Tritium sativum*) was collected at ear-emergence from field experiments with ^{15}N -labeled fertilizer (approximately 16% enriched in ^{15}N). The plant material was mixed in the ratio 100:6 with approximately 200 g of fine quartz sand (0.3–0.4 mm diameter). These mixtures were then composted and sampled according to the procedures given by Knicker and Lüdemann (1995). The C, H, N contents of the plant and compost samples were determined by standard elemental analysis. The elemental compositions are listed in Table 1. On most samples fungi started growing after an incubation time of some weeks. For several samples these were separated from the starting mixture and freeze-dried. The mixed algal material was grown on a nutrient solution containing ^{15}N -enriched potassium nitrate as described previously by Zeliber et al. (1988).

2.1.2. Sediment samples (peat and algal sapropel)

The samples of an algal sapropel used in this study were collected from Mangrove Lake, Bermuda at sediment depths of 0.24, 5.1 and 9.7 m and are described by Hatcher

Table 1
Elemental composition of plant material and the carbon and nitrogen loss that occurs during microbial degradation in composts

Sample	C (%) w/w	H (%) w/w	N (%) w/w	C-loss (%)	N-loss (%)	H/C w/w	C/N w/w
Plant material							
<i>Lolium perenne</i>	38	5.4	5.2	0	0	0.14	7
Wheat	42	5.9	1.2	0	0	0.15	35
Composts							
^{15}N - <i>Lolium perenne</i> (541 days) * *	20	3.0	2.5	75	88	0.15	8
^{15}N -Wheat (631 days) *	29	4.2	5.0	83	27	0.14	6
^{15}N -Wheat (631 days) * *	36	5.0	2.7	81	36	0.14	13

* 60% Water holding capacity; * * water saturation conditions, w/w weight ratios.

Table 2

Elemental composition of sediment samples:

Sample	C (%) w/w	H (%) w/w	N (%) w/w
Torreblanca peat (Spain) (80–100 cm)	35.1	–	1.8
Torreblanca peat (Spain) (100–200 cm)	44.3	–	1.8
Algal sapropel Mangrove Lake (Bermuda) 0.24 m	26.9	4.5	2.1
Algal sapropel Mangrove Lake (Bermuda) 5.10 m	35.1	5.6	2.8
Algal sapropel Mangrove Lake (Bermuda) 9.70 m	42.5	6.6	3.4

et al. (1982a,1983), Spiker and Hatcher (1984) and Orem et al. (1987). The peat samples derive from a Torreblanca peat (Spain) and were collected at a sediment depth of 0.8 to 1 m and 1 m to 2 m (Almendros et al., 1981). The C, H, N contents of sediment samples were determined by standard elemental analysis and are listed in Table 2.

2.1.3. Coal samples

Some of the coal samples used in this study were obtained from the Penn State Coal Sample Bank and Data Base and have been characterized thoroughly by standard chemical methods (Glick and Davis, 1991). Other samples of Torbanite (algal coal) and coalified wood were obtained from locations listed in Table 3 and have been described by Hatcher et al. (1992) and Mukhopadhyay and Hatcher (1993).

2.2. NMR-methods

Solid-state ^{13}C NMR spectra were obtained either with a Bruker MSL-100 spectrometer at a resonance frequency of 25.2 MHz with magic angle spinning at 4 kHz or with a Chemagnetics M-100 spectrometer at a frequency of 25.04 MHz with magic-angle spinning at 3.5 kHz. The cross polarization magic-angle spinning (CPMAS) technique was used (Schaefer and Stejskal, 1976). The ^{13}C -chemical shifts were referenced to tetramethylsilane (0 ppm). A single Hartmann–Hahn contact time of 1 ms was used for all spectra. A repetition time of 5 s was used for the undegraded plant material while repetition times of 300 ms and 100 ms were sufficient for the composted material and coals, respectively.

The solid-state ^{15}N CPMAS NMR spectra were obtained either with a Bruker MSL-300 spectrometer at a resonance frequency of 30.4 MHz and with a magic-angle spinning speed of 4.5 kHz or with a Chemagnetics CMC-300 spectrometer at a frequency of 30.2 MHz using the CPMAS technique with a magic-angle spinning speed of 3.6 kHz. The chemical shifts are referenced to nitromethane nitrogen (0 ppm). The polarization transfer time, T_{NH} , and the proton spin lattice relaxation time in the rotating frame, $T_{1\rho\text{H}}$, were determined from a series of spectra collected at various Hartmann–Hahn-contact times, t . The proton spin lattice relaxation, $T_{1\text{H}}$, was determined indirectly by a special pulse sequence reported by Dev et al. (1987). A more detailed discussion of the relevant relaxation times and their influence upon the relative ^{15}N signal intensities is given by Knicker et al. (1996). The routine solid-state ^{15}N NMR spectra of the undegraded plant material, the plant composts and the sediments were obtained using a

Table 3
Location, stratigraphy, elemental composition and rank of the coal samples studied

Sample	Location	Formation	Age	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Rank	Ref.
Torbanite	South Africa	Karoo System	Permian	46.5	4.5	1.3	ND	–
Coalified wood	Culpeper Virginia USA	Midland Formation	Jurassic	82.7	6.2	1.3	high volatile C bituminous (hvCb)	Hatcher et al., 1992
PSOC 1362	Lawrence County, Pennsylvania, USA	Allegheny Group, Freeport Formation	Middle Pennsylvanian	84.0	6.0	2.0	high volatile A bituminous (hvAb)	Glick and Davis, 1991
PSOC 1517	Mahoning County, Ohio, USA	Allegheny Group, Kittanning Formation	Middle Pennsylvanian	83.6	5.4	1.9	high volatile B bituminous (hvBb)	Glick and Davis, 1991
PSOC 1382	Moffat County, Colorado, USA	Mesaverde Group Williams Fork Formation	Late Cretaceous	75.8	5.0	2.0	subbituminous A (SubA)	Glick and Davis, 1991

ND – not determined.

PSOC – Penn State Coal Sample Bank.

contact time of 0.7 ms. The solid-state ^{15}N NMR spectra of the coals were obtained with a contact time of 1 ms. For the undegraded plant material a repetition time of 5 s was applied, whereas for the composted material a repetition time of 300 ms was sufficient. The sediment and coal solid-state ^{15}N NMR spectra were obtained with a repetition time of 100 ms after accumulation of 0.5 to 2 million scans.

3. Results and discussion

3.1. NMR spectra and optimization of acquisition parameters

NMR spectroscopy, as applied to organic material in sediments and coals, is primarily employed to determine the gross chemical structure of the organic material and to attempt a quantitative correlation of the different signal intensities to the chemical composition. This is usually accomplished by relating the chemical shifts and intensities of peaks to known structural elements. However, before one can relate peak intensities to quantifiable structural elements, it is necessary to demonstrate that the NMR spectra have been obtained under optimal conditions. Even if optimization is achieved, there are unavoidable conditions which cause deviation from quantitative signal representation. The appropriate conditions for solid-state ^{13}C NMR spectroscopy have been addressed by Wilson (1987).

To establish the optimum instrumental conditions for both solid-state ^{13}C and ^{15}N NMR spectroscopy of complex materials such as coal and related precursors, it is imperative that one demonstrates, by measurements of spin dynamics, that the proper conditions for quantitative spectroscopy are used. Thus, the discussion below focuses on the various experiments conducted to establish that both ^{13}C and ^{15}N NMR spectra of samples examined are as quantitative as possible. Before discussing the various experiments it is appropriate to mention the spectral assignments for the many peaks commonly observed in ^{13}C and ^{15}N NMR spectra of coal and related plant precursors. Tables 4 and 5 contain possible assignments of signals commonly observed in both the

Table 4

Assignments for the peaks in solid-state ^{13}C NMR spectra (referenced to tetramethylsilane = 0 ppm) (Wilson, 1987; Knicker et al., 1995a)

Chemical shift range (ppm)	Assignment
220–160	Carboxyl/carbonyl/amide carbons
160–140	Aromatic COR or CNR groups
140–110	Aromatic C–H carbons, guaiacyl C-2, C-6 in lignin, olefinic carbons
110–90	Anomeric carbon of carbohydrates, C-2, C-6 of syringyl units of lignin
90–60	Carbohydrate-derived structures (C-2 to C-5) in hexoses, C- α of some amino acids, higher alcohols
60–45	Methoxyl groups and C-6 of carbohydrates and sugars, C- α of most amino acids
45–25	Methylene groups in aliphatic rings and chains,
25–0	Terminal methyl groups

Table 5

Assignments for peaks in the solid-state ^{15}N NMR spectra (referenced to nitromethane = 0 ppm) (Martin et al., 1981; Witanowski et al., 1993)

Chemical shift range (ppm)	Assignment
25 to -25	Nitrate, nitrite, nitro groups
-25 to -90	Imine, phenazine, pyridine, Schiff-bases
-90 to -145	Purine, nitrile groups
-145 to -220	Chlorophyll-N, purine/pyrimidine, imidazole, substituted pyrroles
-220 to -285	Amide/peptide, N-acetyl derivatives of amino sugars, tryptophane, proline, lactams, unsubstituted pyrroles, indoles and carbazoles
-285 to -325	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, aniline derivatives)
-325 to -350	free amino groups in amino acids and sugars
-350 to -375	NH_4^+

^{13}C and ^{15}N NMR spectra, respectively, of plant materials, coals and related substances. As an example, both the ^{15}N and ^{13}C NMR spectra of the ^{15}N -enriched wheat compost incubated for 631 days are shown in Figs. 1 and 2, respectively. The assignments are discussed below as they relate to specific samples.

Solid-state NMR spectra of sediments and coals are usually obtained with the cross polarization magic-angle spinning (CPMAS) technique (Schaefer and Stejskal, 1976) after simultaneously irradiating with two radio frequency fields during the Hartmann–Hahn contact time t . In such an experiment the magnetization is generally transferred from the proton spin system with a high natural abundance (^1H 's) and short spin–lattice relaxation times to the dilute (i.e. ^{13}C or ^{15}N) spin system with low natural abundance and long spin–lattice relaxation time. Following the polarization transfer, enhanced signals of the dilute-spin nuclei are observed. The intensity I of a signal in a CPMAS NMR spectrum is a complex function of three relaxation or polarization transfer times.

$$I_t = I_0(T_{1\text{H}}, T_{\text{XH}}, T_{1\rho\text{H}}, t) \quad (1)$$

I_t is the intensity observed after the Hartmann–Hahn-contact time t . I_0 is directly proportional to the number of nuclei in the sample, $T_{1\text{H}}$; the proton spin lattice relaxation

Fig. 1. Solid-state ^{13}C NMR spectrum of a compost of ^{15}N -enriched wheat, incubated for 631 days at a sample moisture of 60% water-holding capacity (WHC). Typical chemical shift ranges are shown.

Fig. 2. Solid-state ^{15}N NMR spectrum of a compost of ^{15}N -enriched wheat, incubated for 631 days at a sample moisture of 60% WHC. Typical chemical shift ranges are shown. Asteriks indicate spinning side bands.

time, T_{XH} ; the cross polarization time for the dilute-spin system, $T_{1\rho\text{H}}$; and the proton spin lattice relaxation time in the rotating frame; t is the contact time.

Quantitative solid-state CPMAS NMR spectra can only be obtained if saturation effects are avoided. This is accomplished by ensuring that the time span between pulses is approximately five times larger than the longest proton spin lattice relaxation time, $T_{1\text{H}}$, observed for the nuclei in the sample under study (Wilson, 1987). To ensure that each signal observed in a spectrum is proportional to the number of carbons or nitrogens it represents, it is imperative that sufficient time be allowed for spin-lattice relaxation of the protons associated with the carbon or nitrogen of a particular peak. Therefore, $T_{1\text{H}}$'s of several peaks assigned to different functional groups in the solid state ^{13}C and ^{15}N NMR spectra were determined for both the undegraded and composted plant material (Table 6). Fig. 3 shows the variation of $T_{1\text{H}}$ for two notable peaks as a function of incubation time for wheat compost. The peak at 72 ppm in the solid-state ^{13}C NMR spectra is assigned to carbohydrate-like carbons and the signal at -257 ppm in the solid state ^{15}N NMR spectra is assigned to amide nitrogens in proteinaceous structures (Knicker, 1993). An interesting observation is that for all the signals in both the ^{13}C and the ^{15}N NMR spectra, $T_{1\text{H}}$ decreases with prolonged degradation of the plant material. After two years of incubation the $T_{1\text{H}}$ values for all signals are less than 58 ms. This can be explained by either an increase in radicals which efficiently shorten $T_{1\text{H}}$ or by structural changes occurring during composting. These results suggest that, for degraded plant material assumed to be characteristic of peat and coals, the pulse delay between single scans has to be five times $T_{1\text{H}}$ and therefore can be shorter than 300 ms ($5 \times 58 \text{ ms} = 290 \text{ ms}$).

Solid-state ^{15}N NMR spectra of soils and sediments with a nitrogen content of less than 2% require accumulation of more than 0.5 million scans to obtain acceptable signal-to-noise (S/N) ratios. By applying a pulse delay of 300 ms, the measurement time for such spectra is still several days. In a previous study (Knicker, 1993), solid-state ^{15}N NMR spectra of soil fractions were obtained with two repetition times, one of 300

Table 6

 T_{1H} (ms) in ^{13}C and ^{15}N CPMAS NMR experiments determined from several compost samples

ppm	^{13}C CPMAS (25.2 MHz)						^{15}N CPMAS (30.4 MHz)		
	172	153	128	104	72	32	-257	-306	-346
^{15}N - <i>Lolium perenne</i> , water saturation conditions									
0 days	155	—	129	266	260	162	219	236	285
7 days	76	—	90	86	75	112	144	221	74
24 days	27	—	46	51	41	67	63	58	44
248 days	25	—	24	20	22	45	40	42	36
541 days	25	—	25	26	30	50	58	—	55
^{15}N -Wheat, 60% WHC									
0 days	372	399	402	427	411	382	450	416	760
29 days	96	—	89	160	148	68	82	103	78
58 days	53	62	65	102	122	49	63	—	48
179 days	29	29	24	42	43	31	—	—	—
255 days	18	20	16	33	35	22	33	44	32
631 days	13	13	12	25	27	17	42	—	26
^{15}N -Wheat, water saturation conditions									
58 days	35	—	—	95	92	44	85	89	99
179 days	35	62	57	83	81	50	—	—	—
255 days	19	29	26	75	75	27	44	51	33
631 days	19	20	18	33	35	24	35	38	37

ms and the other of 100 ms. Comparison of the relative intensity distributions for peaks in these two spectra showed no differences outside the experimental error. Therefore, for routine solid-state ^{15}N NMR spectra of sediments and coals with natural ^{15}N abundance,

Fig. 3. Spin-lattice-relaxation times T_{1H} of the signals at 72 ppm (carbohydrates) in the solid-state ^{13}C and -257 ppm (amides) in the solid-state ^{15}N NMR spectra of composts of ^{15}N -enriched wheat (60% WHC) as function of incubation time.

Fig. 4. Intensity of the signals at -257 ppm (amides) and -346 ppm (free amino groups) in the solid-state ^{15}N NMR spectrum of a compost of ^{15}N -enriched wheat (631 days, 60% WHC) as a function of contact time (reproduced from Knicker et al., 1996).

a repetition time of 100 ms was used. We realize that such a short repetition time might induce saturation of certain peaks and lead to non-quantitative representation; however, we are limited by time constraints and assume that the chosen repetition time will not influence our data significantly.

The efficiency of magnetization transfer from protons to a group of dilute nuclei X (^{13}C and ^{15}N) in a CPMAS experiment depends upon the local proton concentration, and on the rotational correlation time of the X-nucleus. Only if $T_{\text{XH}} \ll t \ll T_{1\rho\text{H}}$ can quantitative results be obtained from the CPMAS NMR spectrum (Wilson, 1987). Although several studies have already examined the cross polarization dynamics for solid-state ^{13}C NMR of soil organic matter, peat and coals (Wilson, 1987; Fründ and Lüdemann, 1989a, Fründ and Lüdemann, 1989b), only a few investigations of these parameters have been reported for solid-state ^{15}N NMR (Ripmeester et al., 1986; Almendros et al., 1991; Derenne et al., 1993; Knicker et al., 1995a, 1996). Thus, it was necessary to examine the spin dynamics for the ^{15}N NMR system for the particular set of samples under investigation.

In this study T_{NH} and $T_{1\rho\text{H}}$ in a ^{15}N NMR experiment were estimated from a determination of the spectral intensities I as function of t obtained from ^{15}N -enriched undegraded plant, fungal material and plant composts. Fig. 4 shows the relationship between signal intensity I for two major ^{15}N NMR peaks in the composted wheat and the contact time t (Knicker et al., 1996). The steep initial increase of I at short t and the rather slow diminution of I at longer t suggests that $T_{\text{NH}} \ll T_{1\rho\text{H}}$. By fitting these curves as described by Wilson (1987), we can calculate both T_{NH} and $T_{1\rho\text{H}}$. Within the precision of the intensities measured, each curve can be described by one T_{NH} and one $T_{1\rho\text{H}}$. Furthermore an optimum average value of t can be obtained for use in the NMR experiments, one which would provide a quantitative representation of all the different signals in the spectra even though each signal may have a slightly different optimal value of t (Table 7). This optimal value is a t , at which the intensity loss of every signal of the different compounds is similar. Comparison of the data shown in Table 7 indicates that the best value for t in composted plant material is $t = 0.7$ ms (Knicker, 1993).

Due to the low local proton density of typical coalified material which might contain

Table 7

Cross polarization parameters determined for a mixed fungal culture, *Lolium perenne* and several plant composts

Signal (ppm)	$T_{1\rho\text{H}}$ (ms)	T_{NH} (ms)	t_{optimal} (ms)
<i>mixed fungi</i>			
– 256	5.7	0.11	0.8
– 306	4.6	0.06	0.5
– 345	15.4	0.34	1.2
<i>Lolium perenne</i>			
– 256	5.8	0.23	0.8
– 306	5.5	0.09	0.3
– 345	6.9	0.30	1.0
<i>Lolium perenne</i> compost, 248 days, water saturation conditions			
– 257	6.2	0.10	0.4
– 306	6.3	0.06	0.3
Wheat compost, 60% WHC			
– 207	4.9 *	0.15 *	0.5 *
– 257	4.5 *	0.16 *	0.5 *
– 306	4.8 *	0.08 *	0.3 *
– 345	6.6 *	0.29 *	0.8 *
Wheat compost, water saturation conditons			
– 257	4.5 *	0.13 *	0.4 *
– 306	3.5 *	0.06 *	0.4 *
– 346	5.0 *	0.19 *	0.7 *

* averaged values from several composts.

structures with nitrogens both adjacent to and remote from protons, both pyridinic N and pyrrolic N for example, magnetization transfer is expected to be less efficient than in compost samples which typically contain relatively more protons. Therefore, for the solid-state ^{15}N NMR spectra of coal, a contact time of 1 ms was used, allowing extra time for polarization transfer. Previously it was shown that, for ^{15}N -enriched pyridine and pyridinium ions sorbed on coal, the ^{15}N NMR signal intensities were virtually independent of contact times between 1 and 5 ms (Ripmeester et al., 1986). Thus, a contact time of 1 ms appears to be appropriate for coal.

In order to test whether $t = 1$ ms was long enough to observe all heterocyclic compounds in the coal samples, especially those remote from a directly attached proton with which they can be effectively polarized, solid-state ^{15}N NMR spectra of a high volatile A bituminous coal (PSOC 1362) were obtained at $t = 1$ ms and $t = 2.5$ ms (Fig. 5). In both spectra the main signal is at -240 ppm with a downfield shoulder at -175 ppm. While the signal at -240 ppm, assigned to protonated pyrrolic N, decreases with increasing t , the signal at -175 ppm, assigned to nonprotonated pyrrolic N, shows virtually no intensity loss. Signals in the region of nonprotonated pyridinic structures (-40 to -90 ppm) were not identified. These results indicate that a long t of 2.5 ms only results in a signal intensity loss for nitrogen compounds suspected of having strong

Fig. 5. Solid-state ^{15}N NMR spectra of a high volatile A bituminous coal (PSOC 1362) obtained at $t = 1$ ms and $t = 2.5$ ms (reproduced from Knicker et al., 1995a).

dipolar interactions (protonated N). Thus, a shorter t is better in that it recovers the signal for protonated N's while providing sufficient time for the polarization of nonprotonated N's. It is possible that pyridinic nitrogens are not observed because they require longer polarization times and their $T_{1\rho\text{H}}$ values are short such that the conditions necessary for quantitative signals are not met ($T_{\text{NH}} \ll t \ll T_{1\rho\text{H}}$). On the basis of our unpublished studies of model pyridinic polymers (polyvinylpyridine), we do not believe that the above explanation is applicable for the lack of pyridinic nitrogen in coal. Rather, we believe that the lack of pyridinic nitrogen reflects its scarcity in coal.

3.2. Undegraded plant material and plant composts

Coal originates from plant remains, algal and fungal residues or microbial material in peat that are altered during maturation or coalification. In order to understand these processes, it is important to understand the chemical composition of the undegraded precursors.

Fig. 6 shows the solid-state ^{13}C and ^{15}N NMR spectra of *Lolium perenne*, beech wood, algal material and fungal material. The solid-state ^{13}C NMR spectra of the *Lolium* and the wood sample have intense signals in the chemical shift region between 110 and 60 ppm; these originate mainly from cellulosic and hemicellulosic structures. The acetyl group of some acetylated hemicellulosic sugar derivatives contributes to the signal intensity in the chemical shift region of aliphatic structures between 45 and 0 ppm. This signal can be clearly identified in the spectrum of the beech sawdust. In the same spectrum, the signal between 220 to 160 ppm is most likely assigned to the carboxylic-C of uronic acids in hemicelluloses.

Fig. 6. Solid-state ^{15}N and ^{13}C NMR spectra of ^{15}N -enriched undegraded plant and ^{15}N -enriched microbial materials. Asteriks indicate spinning sidebands.

Another important component of higher plants, lignin, can be identified in the solid-state ^{13}C NMR spectra of plant material in the aromatic chemical shift region between 160 and 110 ppm. Propanyl side-chains of lignin contribute to the signal intensity in the chemical shift region between 50 and 85 ppm, but these are usually masked by the intense peaks for carbohydrates. The methoxyl groups of guaiacyl and syringyl units in lignin give a narrow signal at 56 ppm (Lüdemann and Nimz, 1973; Hatcher et al., 1982b). This signal can be clearly identified in the solid-state ^{13}C NMR spectrum of the beech sawdust.

The solid-state ^{13}C NMR spectrum of the untreated grass (*Lolium perenne*) material shows higher relative intensities in the chemical shift region from 220–160 ppm and from 45–0 ppm compared to the solid-state ^{13}C NMR spectrum of wood. These peaks can originate from the high content of paraffinic and ester structures of cutins present typically in grasses. The high total nitrogen content of *Lolium perenne* (approximately 5%) and its low C/N ratio of 7 (Table 1) are consistent with the fact that more than 80% of the total relative signal intensity of its ^{15}N NMR spectrum (Fig. 6, Table 8) can be assigned to amide structures. This further leads to the conclusion that most of the

Table 8

Relative signal intensity of various peaks in the solid-state ^{13}C and ^{15}N NMR spectra of plant material and a compost

^{13}C CPMAS (25.2 MHz)						^{15}N CPMAS (30.4 MHz)				
chemical shift range	220/160	160/110	110/60	60/45	45/0	25/-25	-145/-220	-220/-285	-285/-325	-325/-375
Fresh plant										
<i>Lolium</i>	10.0	10.6	50.2	7.9	21.3	1.4	2.6	83.1	8.3	4.6
Beech wood	1.6	14.9	74.8	5.7	3.0	–	–	–	–	–
Compost										
<i>Lolium</i> (541 days)	14.8	14.9	20.6	9.1	35.9	0	5.1	86.0	6.6	2.2

signal intensity in the region from 220–160 ppm in the ^{13}C NMR spectrum originates from amide-C which overlaps with the signal from the carboxylic-C. Assuming that, in proteins and peptides every amide-C can be assigned to two aliphatic-C, the signal in the region from 45–0 ppm in the ^{13}C NMR spectrum most probably originates mostly from the aliphatic side chains of peptides rather than from paraffinic structures of plant waxes (Knicker and Lüdemann, 1995).

The solid-state ^{13}C NMR spectra for algal and fungal material show resonance lines in the aromatic region (160–110 ppm) that are most likely assigned to aromatic carbons in amino acids such as phenylalanine, tyrosine or histidine. The main intensity in these spectra is found in the carboxylic and aliphatic region, which is not astonishing due to the well known high protein content of such samples.

The solid-state ^{15}N NMR spectra of all undegraded materials (Fig. 6) are similar with a dominant signal at –257 ppm, the chemical shift region (–220 to –285 ppm) of mostly amide-like structures which include proteins, acetylated amino sugars, and lactams. Unsubstituted pyrroles normally give a signal at –235 ppm which overlaps that of the broad amide resonance line. Indoles and carbazoles may contribute to the signal intensity in the chemical shift region between –230 to –280 ppm. Had the pyrroles, indoles and carbazoles been major components, the broad peak would be shifted towards lower field. The peaks at –294 ppm and –306 ppm may originate from NH_2 groups in basic amino acids or in nucleosides (Knicker et al., 1994). Another distinct signal can be identified at –346 ppm and is assigned to free aliphatic amino groups of amino acids and sugars (–325 to –350 ppm). The ring-N in histidine or nucleic acid derivatives as well as porphyrin-N and substituted pyrrolic-N structures contribute to the signal in the region between –145 and –220 ppm.

Fig. 7 shows the solid-state ^{13}C and ^{15}N NMR spectra of a compost consisting of ^{15}N -labeled *Lolium perenne* incubated for 541 days under water saturation conditions. This experiment was performed to simulate the natural degradation of plant matter that would be expected to predominate during the early stages of peatification. There are significant quantitative differences between the solid-state ^{13}C NMR spectra of the undegraded and the degraded plant material. The largest intensity loss occurs in the chemical shift range between 110 and 60 ppm (Table 8). These resonances are attributable to carbohydrates and they reflect the fact that the predominant reactions

Fig. 7. Solid-state ^{15}N and ^{13}C NMR spectra of a plant compost of ^{15}N -enriched *Lolium perenne* incubated under water saturation conditions after 541 days of incubation. Asteriks indicate spinning sidebands.

occurring with increasing degradation are hydrolysis and removal of carbohydrates. This confirms other studies (Bates and Hatcher, 1989; Inbar et al., 1989; Almendros et al., 1991) that show the selective degradation of labile carbohydrates during the early stages of maturation of organic matter. In all other chemical shift ranges in the spectra an apparent relative increase in peak height is seen.

The solid-state ^{15}N -NMR spectrum of decomposed ^{15}N -labeled *Lolium perenne* still shows its main signal intensity in the chemical shift region assigned to amides. This feature is surprisingly similar to that of the undegraded plant material. The result of this experiment supports other previous studies which showed that during microbial degradation, nitrogen occurs mainly in amides (Almendros et al., 1991; Knicker and Lüdemann, 1995). This finding is quite surprising, since amide functional groups are expected to decompose rapidly in the presence of soil microbes. The stabilization may be due to the fast turnover of nitrogen from dead to growing microbial biomass but can also be explained by refractory amide structures. Evidence for such refractory amides was recently reported by Knicker et al. (1991, 1995b), Derenne et al. (1993). Interestingly, no peaks were observed in the chemical shift region of pyridinic nitrogen. If one subscribes to the theory that nitrogen-containing structures in peat derive from polymerization/condensation reactions of lignin degradation products with ammonia produced during degradation (Flaig et al., 1975), then one would expect to observe pyridinic nitrogen. The lack of pyridinic nitrogen severely constrains this theory.

3.3. Sediments

All of the above mentioned ^{15}N CPMAS NMR studies involving ^{15}N -enriched plant and compost materials were initiated to learn more about the chemistry of nitrogen during peatification and early diagenesis. While a major fraction of native peat is several hundred to several hundred thousand years old, the laboratory produced material, studied here, has only been incubated for 2 yr. To understand the fate of nitrogenous substances in natural peats that eventually become coal, ^{15}N NMR studies of some recent and fossil sediments were initiated. The samples varied from a humic peat, representing vascular plant deposits, to an algal sapropel which represents peatified algal material. Fig. 8 shows the solid-state ^{15}N NMR spectra of two horizons of a peat (Torreblanca peat, Spain) (Almendros et al., 1981; Knicker et al., 1995b), and Fig. 9 shows the ^{15}N NMR spectra of three horizons of an algal sapropel from Mangrove Lake, Bermuda (Hatcher et al., 1982a, 1983). All spectra show mostly a large broad peak at -257 ppm which is most likely due to amide nitrogen. The spectra have comparable features to the undegraded plant, algal and fungal material and to the ^{15}N -enriched composts in Figs. 2, 6 and 7. Only a small peak and/or shoulder can be identified in the region of imidazoles and pyrroles (-145 to -240 ppm). Even in these spectra of aged sedimentary organic matter signals are not observed in the region of pyridinic nitrogen. If we consider that the resonance region between -145 to -240 ppm represents mostly pyrrole or imidazoles and that some indoles may be hidden by the broad resonance at -257 ppm, it can be concluded that heterocyclic aromatic N cannot contribute more than 10–15% of the total nitrogen signal intensity. These results support the conclusions obtained from previous ^{15}N NMR studies involving incubation experiments and soils (Knicker et al.,

Fig. 8. Solid-state ^{15}N NMR spectra of a peat (Torrealblanca peat, Spain) obtained from a sediment depth at 80–100 cm and 100–200 cm (Almendros et al., 1981; Knicker et al., 1995b). Asteriks indicate spinning sidebands.

1993) that some amide functional groups resist microbial degradation and can be preserved even in aged sediments. It is particularly noteworthy that the lowermost horizon of the algal sapropel displays an ^{15}N NMR spectrum showing mostly amide nitrogen when previous studies by Hatcher et al. (1983) indicated that a significant amount of degradation of labile material has occurred at this horizon. This indicates that the amide structures present must be extremely resistant to mineralization.

Fig. 9. Solid-state ^{15}N NMR spectra of an algal sapropel (Mangrove Lake, Bermuda) obtained from sediment depth at 0.24 m, 5.1 m and 9.7 m. Asteriks indicate spinning sidebands.

3.4. Coal samples

Fig. 10 compares the solid-state ^{13}C and ^{15}N NMR spectra of a Torbanite, coalified wood of high volatile C bituminous coal rank (see Hatcher et al., 1992; Knicker et al., 1995a), a subbituminous A coal (PSOC 1382) and a high volatile B bituminous coal (PSOC 1517). Although Torbanite is derived from algal residues, the coalified wood and coal samples originate mostly from lignin-rich plant precursors that impart most of the aromatic structural components to the coal. The solid-state ^{13}C NMR spectrum of the Torbanite shows a broad subordinate signal in the chemical shift region of aromatics and a dominant resonance in the aliphatic region. The latter resonance, peaking at 30 ppm, indicates the presence of polymethylenic carbons derived most likely from resistant algaenans (Largeau et al., 1986). The intensity between 160 and 110 ppm originates from aromatic structures but are probably not original structures and are most likely formed by cyclization and condensation of aliphatic structures during coalification. However, the incorporation of aromatic structures from lignin-derived components admixed in the Torbanite cannot be ruled out.

The solid-state ^{15}N NMR spectra in Fig. 10 are substantially different from all solid-state ^{15}N NMR spectra presented before for fresh plant materials and sedimentary materials. The coalified or matured samples produce ^{15}N NMR spectra with the main peak in the chemical shift region between -120 and -270 ppm, peaking at -240 ppm. This resonance is most likely that of pyrrolic, imidazolic and indolic nitrogen. Because

Fig. 10. Solid-state ^{15}N and ^{13}C NMR spectra of Torbanite, coalified wood and various coals.

amides give signals in the same approximate chemical shift region they cannot be distinguished from the broad main signal and, thus, may contribute to the signal. However, as mentioned above, a dominance of amide-N induces a peak maximum at -257 ppm whereas a dominance by pyrrolic structures shifts the peak maximum to -240 ppm. The broadness of the resonance line indicates the presence of a large number of different structures, perhaps reflecting extensive substitution and crosslinking of the pyrrolic ring system or perhaps contributions from amides. The solid-state ^{15}N NMR spectra of the PSOC 1382 and PSOC 1571 coals show an additional narrow peak at -345 ppm that is due to the added reference glycine. The broad shoulder at the low field side (-120 to -210 ppm) of the broad peak at -240 ppm may originate from pyrrolic nitrogens in which the lone pair electrons have been delocalized by substitution. Unsubstituted indolic or carbazolic nitrogens show higher electron density and, therefore, a higher magnetic shielding as compared to nitrogen in pyrroles. Resonances from carbazole and indole nitrogens may contribute to the high field shoulder (-230 to -280 ppm) of the broad peak.

It is generally believed that nitrogen in coal occurs not only in pyrrolic, but also in pyridinic structures. Unsubstituted pyridinic N was found to give signals in the chemical shift region between -40 to -90 ppm (Ripmeester et al., 1986; Witanowski et al., 1993). Only the Torbanite spectrum shows a weak signal at -80 ppm which may be assigned to such pyridinic-N. In the solid-state ^{15}N NMR spectra of the coals, presented here, signals cannot be distinguished from the noise in the chemical shift assigned to unsubstituted pyridinic-N. However, it has to be considered that less sufficient cross-polarization transfer may occur for the unsubstituted pyridinic-N, and this can result in intensity losses.

Strong acidic moieties such as carboxylic acid functional groups on condensed aromatic ring systems may induce protonation or N-alkylation of pyridinic nitrogen. This would lead to a dramatic increase of its magnetic shielding of up to 100 ppm or more in a ^{15}N NMR spectrum. However, this would require a complete proton transfer from acidic groups with $pK_a > 4$ to the pyridinic-N (Johnson and Rumon, 1965). Evidence for such a proton transfer by strong acidic carboxyl functional groups in oxidized coal (Illinois No. 6) samples was previously shown to give rise to a ^{15}N NMR signal at -150 ppm (Ripmeester et al., 1986). The ^{13}C NMR spectra of the coals, however, does not show signals in the chemical shift region of carboxylic acid functional groups. Thus, pyridinium ions that are caused by carboxylic acid functional groups are not expected to be present in the coal samples presented here. On the other hand Kelemen and Kwiatek (1995) recently suggested the presence of pyridinium ions related to solid-state pyridine-phenol interactions. Such compounds could contribute to the signal intensity around -150 ppm. Their presence cannot be completely excluded.

4. Summary and conclusions

Solid-state ^{15}N NMR is shown to be an emerging technique for the examination of nitrogen structures in coal and precursor plant material. Using ^{15}N -labeled plant material to determine important NMR spectroscopic acquisition parameters, quantitative solid-

state ^{15}N NMR spectra with an acceptable S/N ratio were obtained for samples having natural-abundance ^{15}N , after accumulation of 0.5–2 million scans at a contact time between 0.7 and 1 ms and a pulse repetition time of 100 ms. The optimized parameters were applied to several organic sediments, coalified wood, Torbanite, and three coals from the Penn State Coal Sample Bank (Glick and Davis, 1991).

From these preliminary analyses, it was observed that, during early diagenesis of organic sediments, nitrogen functional groups in degrading plants and sediments are most likely amides, which are biogenic remnants that survive chemical hydrolysis and early diagenesis, perhaps because they are sterically or physically protected. In more mature organic remains or coals, the nitrogen-containing structures appear to be mostly pyrroles, imidazoles and indoles. With the exception of the solid-state ^{15}N NMR spectrum of the Torbanite, the spectra are generally devoid of pyridinic structures. Assuming that cross polarization dynamics do not remove completely the pyridinic signal in the ^{15}N spectra (see discussion above) and considering the low S/N ratio of the spectra and a possible intensity loss due to inefficient cross polarization, the results of this study suggest that pyridinic-compounds contribute less than 25% to the total nitrogen of the samples. This contrasts sharply with recent studies obtained with X-ray absorption near-edge structure spectroscopy (XANES) which suggest a pyridinic nitrogen content of 47% and pyrrolic nitrogen content of 53% (Kirtley et al., 1993). On the other hand, the results obtained here are consistent with those obtained from X-ray photoelectron spectroscopy (XPS) (Burchill and Welch, 1989, Wójtowicz et al., 1995) where it was found that the pyrrolic form predominates in bituminous coal and that the proportion of pyridinic nitrogen increases with rank. This suggests that six-membered heterocyclic structures are more stable in the later stages of coalification.

This NMR investigation has demonstrated that, despite the low natural abundance of the ^{15}N isotope and the low NMR sensitivity, solid-state ^{15}N NMR can be applied to organic sediments and whole coal samples. Although much work is still necessary with respect to the question of quantification, solid-state ^{15}N NMR spectroscopy may add substantially to the understanding of the nature and diagenesis of nitrogen structures in coals.

Acknowledgements

Financial support was obtained from the Department of Energy. The Microanalysis Laboratory of the University of Regensburg is gratefully acknowledged for the C/H/N analyses of the samples. Prof. Dr. G. Almendros (CSIC, Madrid, Spain) is gratefully acknowledged for the donation of the Torreblanca peat samples. Prof. Dr. H.-D. Lüdemann (University of Regensburg, BRD) and Dr. A. Benesi (Pennsylvania State University, USA) are gratefully acknowledged for the technical assistance in obtaining the solid-state ^{15}N NMR spectra.

References

- Aho, M.J., Hämäläinen, J.P. and Tummuvaori, J.L., 1993. Conversion of peat and coal nitrogen through HCN and NH_3 to nitrogen oxides at 800°C. *Fuel*, 72: 837–841.

- Almendros, G., Fründ, R., González-Vila, F.J., Haider, K., Knicker, H. and Lüdemann, H.-D., 1991. Analysis of ^{13}C and ^{15}N CPMAS NMR-spectra of soil organic matter and composts. *FEBS Lett.*, 282: 119–121.
- Almendros, G., Polo, A. and Dorado, E., 1981. Características de la materia orgánica en las formaciones turbosas litorales de Torreblanca (Castellón). *Anales Edafol. Agrobiol.*, 40: 223–225.
- Anderson, H.A., Bick, W., Hepburn, A. and Stewart, M., 1989. Nitrogen in humic substances. In: M.B. Hayes, P. MacCarthy, R.L. Malcolm and R.S. Swift (Editors), *Humic Substances II, In Search of Structure*. Wiley-Interscience, Chichester, pp. 223–253.
- Attar, A. and Hendrickson, G. G., 1982. In: R.A. Meyers (Editor), *Coal Structure*. Academic Press, New York, pp. 155–162.
- Bates, A.L. and Hatcher, P. G., 1989. Solid-state ^{13}C NMR studies of a large fossil gymnosperm from the Yallourn Open Cut, Latrobe Valley, Australia. *Org. Geochem.*, 14: 609–617.
- Benzing-Purdie, L., Ripmeester, J.A. and 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 spectroscopy. *J. Agric. Food Chem.*, 31: 913–915.
- Burchill, P. and Welch, L.S., 1989. Variation of nitrogen content and functionality with rank for some UK bituminous coals. *Fuel*, 68: 100–104.
- Cheshire, M.V., Williams, B.L., Benzing-Purdie, L., Ratcliffe, C.J. and Ripmeester, J.A., 1990. Use of ^{15}N NMR spectroscopy to study transformations of nitrogenous substances during incubation of peat. *Soil Use Management*, 6: 90–92.
- Derenne, S., Largeau, C. and 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 ultralaminae-containing kerogens. *Geochim. Cosmochim. Acta*, 57: 851–857.
- Dev, S.B., Burum, D.P. and Rha, C.K., 1987. High resolution CPMAS and solution NMR studies of polysaccharides. Part 1: starch. *Spectrosc. Lett.*, 20: 853–869.
- Flaig, W.J.A., Beutelspacher, H. and Rietz, E., 1975. Chemical composition and physical properties of humic substances. In: J.E. Gieseking (Editor), *Soil Components*. 1. Springer, New York, pp. 1–211.
- Fründ, R. and Lüdemann, H.-D., 1989a. The quantitative analysis of solution and CPMAS C-13 NMR spectra of humic material. *Sci. Total Environ.*, 81/82: 157–168.
- Fründ, R. and Lüdemann, H.-D., 1989b. ^{13}C -NMR-Spectroscopy of lignins and lignocellulosic material. In: A. Chesson and E.R. Ørskov (Editors), *Physico-Chemical Characterization of Plant Residues for Industrial and Feed Use*. Elsevier Applied Science, Brussels, pp. 110–117.
- Glick, D.C. and Davis, A., 1991. Operation and composition of the Penn State coal sample bank and data base. *Org. Geochem.*, 17: 421–430.
- Hatcher, P.G., Simoneit, B.R.T., Mackenzie, F.T., Neumann, A.C., Thorstenson, D.C. and Gerchakov, S.M., 1982a. Organic geochemistry and pore water chemistry of sediments from Mangrove Lake, Bermuda. *Org. Geochem.*, 4: 93–112.
- Hatcher, P.G., Breger, I.A., Szeverenyi, N.M. and Maciel, G.E., 1982b. Nuclear magnetic resonance studies of ancient buried wood: II. Observations on the origin of coal from lignite to bituminous coal. *Org. Geochem.*, 4: 9–18.
- Hatcher, P.G., Spiker, E.C., Szeverenyi, N.M. and Maciel, G.E., 1983. Selective preservation and origin of petroleum-forming aquatic kerogen. *Nature*, 305: 498–501.
- Hatcher, P.G., Lerch, H.E. and Verheyen, T.V., 1989a. Organic geochemical studies of the transformation of gymnospermous xylem during peatification and coalification to subbituminous coal. *Int. J. Coal Geol.*, 13: 65–97.
- Hatcher, P.G., Wilson, M.A., Vassallo, A.M. and Lerch, H.E. III, 1989b. Studies of angiospermous wood in Australian brown coal by nuclear magnetic resonance and analytical pyrolysis: new insights into the early coalification process. *Int. J. Coal Geol.*, 13: 99–126.
- Hatcher P.G., Faulon J.-P., Wenzel K.A. and Cody, G.D., 1992. A structural model for lignin-derived vitrinite from high-volatile bituminous coal (coalified wood). *Energy Fuels*, 6: 813–820.
- Hewitt, L.J., 1966. Sand and water culture methods used in the study of plant nutrition. *Commonw. Agric. Bureau*, pp. 187–199.
- Inbar, Y., Chen, Y. and Hadar, Y., 1989. Solid-state carbon-13 nuclear magnetic resonance and infrared spectroscopy of composted organic matter. *Soil. Sci. Soc. Am. J.*, 53: 1695–1701.

- Johnson, S.L. and Rumon, K.A., 1965. Infrared spectra of solid 1:1 Pyridine–Benzoic acid complexes; the nature of hydrogen bond as a function of the acid–base levels in the complex. *J. Phys. Chem.*, 69: 75–86.
- Kelemen, S.R. and Kwiatek, P.J., 1995. Quantification of organic species on the surface of fresh and reacted Argonne Premium coal. *Energy Fuels*, 9: 841–848.
- Kirtley, S.M., Mullins, O.C., van Elp, J. and Cramer, S.P., 1993. Nitrogen chemical structure in petroleum asphaltenes and coal by X-ray absorption spectroscopy. *Fuel*, 72: 133–135.
- Knicker, H. 1993. Quantitative ^{15}N - und ^{13}C -CPMAS-Festkörper- und ^{15}N -Flüssigkeits-NMR-Spektroskopie an Pflanzenkomposten und Natürlichen Böden. Diss. Univ. Regensburg, Germany.
- Knicker, H. and 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. *Org. Geochem.*, 23: 329–341.
- Knicker, H., Fründ, R., Lüdemann, H.-D., Almendros, G., González-Vila, F.J. and Martín, F., 1991. Characterisation of nitrogen in compost by N-15 NMR. *Humus-utiset*, 3: 313–315.
- Knicker, H., Fründ, R. and Lüdemann, H.-D., 1993. The chemical nature of nitrogen in native soil organic matter. *Naturwissenschaften*, 80: 219–221.
- Knicker, H., Fründ, R. and Lüdemann, H.-D., 1994. N-15-NMR studies of humic substances in solution. In: N. Senesi and T. M. Miano (Editors), *Humic Substances in the Global Environment and Implications on Human Health*. Elsevier, Amsterdam, pp. 501–506.
- Knicker, H., Hatcher, P.G. and Scaroni, A.W., 1995a. Solid-State ^{15}N NMR spectroscopy of coal. *Energy Fuels*, 9: 999–1002.
- Knicker, H., Almendros, G., González-Vila, F.J., Lüdemann, H.-D. and Martín, F., 1995b. ^{13}C and ^{15}N NMR analysis of some fungal melanins in comparison with soil organic matter. *Org. Geochem.*, 23: 1023–1028.
- Knicker, H., Fründ, R. and Lüdemann, H.-D., 1996. Characterization of nitrogen in plant composts and native humic material by natural abundance N-15 CPMAS and -solution spectra. In: M. Nanny, R.A. Minear and J.A. Leenheer (Editors), *NMR Spectroscopy in Environmental Science and Technology*. Oxford University Press (in press).
- Largeau, C., Casadevall, E., Kadouri, A. and Metzger, P., 1984. Formation of *Botryococcus*-derived kerogens. Comparative study of immature Torbanite and of the extant alga *Botryococcus braunii*. *Org. Geochem.*, 6: 327–332.
- Largeau, C., Derenne, S., Casadevall, E., Kadouri, A. and Sellier, N., 1986. Pyrolysis of immature Torbanite and of the resistant biopolymer (PRB A) isolated from extant alga *Botryococcus braunii*. Mechanism of formation and structure of Torbanite. *Org. Geochem.*, 10: 1023–1032.
- Largeau, C., Derenne, S., Casadevall, E., Berkaloff, C., Corolleur, M., Lugardon, B., Reynaud, J.F. and Connan, J., 1990. Occurrence and origin of “ultralaminar” structures in “amorphous” kerogens of various source rocks and oil shales. *Org. Geochem.*, 16: 889–895.
- Lee, M.L., Novotny, M.V. and Bartle, K.D., 1981. *Analytical Chemistry of Polycyclic Aromatic Compounds*. Academic Press, New York, 446 pp.
- Lüdemann, H.-D. and Nimz, H., 1973. Carbon-13 nuclear magnetic resonance spectra of lignins. *Biochem. Biophys. Res. Commun.*, 52: 1162–1169.
- Maillard, L.C., 1917. General reaction between amino acids and sugars: the biological consequences. *C.R. Soc. Biol.*, 72: 599–601.
- Martin, G.J., Martin, M.L. and Gouesnard, J.-P., 1981. ^{15}N NMR Spectroscopy. In: P. Diehl, E. Fluck and R. Kosfeld (Editors), *NMR Basic Principles and Progress*, 18. Springer, Heidelberg.
- Mukhopadhyay, P.K. and Hatcher, P.G., 1993. Composition of coal. In: B.E. Law and D.D. Rice (Editors), *Am. Assoc. Petrol. Geol. Studies in Geology Series, Hydrocarbons from Coal*, 38: 79–118.
- Orem, W.H., Hatcher, P.G., Spiker, E.S., Szeverenyi, N.M. and Maciel, G.E., 1987. Dissolved organic matter in anoxic pore waters from Mangrove Lake, Bermuda. *Geochim. Cosmochim. Acta.*, 50: 609–618.
- Ripmeester, J.A., Hawkins, R.E., MacPhee, J.A. and Nandi, B.N., 1986. On the interaction between pyridine and coal as studied by CP/MAS ^{15}N n.m.r. *Fuel*, 65: 740–742.
- Schaefer, J. and Stejskal, E.O.R., 1976. Carbon-13 nuclear magnetic resonance of polymers spinning at magic angle. *J. Am. Chem. Soc.*, 98: 1031–1032.
- Schnitzer, M., 1985. Nature of nitrogen of humic substances from soil. In: G.R. Aiken, D.M. McKnight, R.L. Wershaw and P. MacCarthy (Editors), *Humic Substances in Soil, Sediment and Water*. Wiley, New York, pp. 303–325.

- Shinn, J.H., 1984. From coal to single-stage and two-stage products: a reactive model of coal structure. *Fuel*, 63: 1187–1196.
- Snape, C.E. and Bartle, K.D., 1985. Further information on defining asphaltenes in terms of average structural properties. Reply to comments. *Fuel*, 64: 427–429.
- Spiker, E.C. and Hatcher, P.G., 1984. Carbon isotope fractionation of sapropelic organic matter during early diagenesis. *Org. Geochem.*, 5: 283–290.
- Stach, E., Mackowsky, M.-Th., Teichmüller, M., Taylor, G.H., Chandra, D. and Teichmüller, R., 1982. *Stach's Textbook of Coal Petrology*, 3rd ed. Gebrüder Borntraeger, Stuttgart, pp. 5–86.
- Tegelaar, E.W., de Leeuw, J.W., Derenne, S. and Largeau, C. 1989. An reappraisal of kerogen formation. *Geochim. Cosmochim. Acta*, 53: 3103–3106.
- Teichmüller, M., 1989. The genesis of coal from the viewpoint of coal petrology. In: P.C. Lyons and B. Alpern (Editors), *Peat and Coal: Origin, Facies, and Depositional Models*. *Int. J. Coal Geol.*, 12: 1–87.
- Wilson, M.A., 1987. *NMR-Techniques and Application in Geochemistry and Soil Chemistry*. Pergamon Press, Oxford, pp. 1–353.
- Witanowski, M., Stefaniak, L. and Webb, G.A., 1993. Nitrogen NMR spectroscopy. In: G. Webb (Editor), *Annual Reports on NMR Spectroscopy* 25. Academic Press, London.
- Wójtowicz, M.A., Pels, J.R. and Moulijn, J.A., 1995. The fate of nitrogen functionalities in coal during pyrolysis and combustion. *Fuel*, 74: 507–516.
- Zeliber, J.L., Jr., Romankiw, L., Hatcher, P.G. and Colwell, R.R., 1988. Comparative analysis of the chemical composition of mixed and pure cultures of green algae and their decomposed residues by ^{13}C nuclear magnetic resonance spectroscopy. *Appl. Environ. Microbiol.*, 54: 1051–1060.