Stabilization of N-compounds in soil and organic-matter-rich sediments—what is the difference?

Heike Knicker

Lehrstuhl für Bodenkunde, Technische Universität München, 85350 Freising-Weihenstephan, Germany

Most of the organic nitrogen in soils and sediments ultimately derives from living organisms where it is mainly present as peptides and amino acids. These biomolecules are considered to have a biologically labile chemical structure and are expected to be quickly mineralized during early stages of organic matter stabilization. In spite of this, nitrogen is still found in aged soils, recent and even fossilized sediments. To elucidate the nature of this recalcitrant nitrogen and the processes that are involved in its formation, solid-state ^{15}N nuclear magnetic resonance (NMR) spectroscopy was recently introduced into geosciences and applied to various environments differing in the origin of their organic matter precursors as well as in chemical and physical conditions of the environment. Results obtained with this approach indicate that survival of peptide-like structures is a ubiquitous phenomenon, although the mechanisms for their stabilization may differ in different ecological systems. However, a conspicuous change in organic nitrogen composition is observed in fossilized sediments and for organic matter formed by vegetation fires. Cyclization and rearrangement of peptide structures result in the formation of heteroaromatic N during fossilization, which was not detected for recent sediments and soils. From this, it may be concluded that such compounds are only formed in environments in which abiotic transformation of biogenic precursors dominates over biotic degradation.

1. Introduction

Nitrogen is a major nutrient element controlling the cycling of organic matter in the biosphere. As a limiting growth factor, the concentration of nitrogen

in both its organic and inorganic forms is closely related to biological productivity in terrestrial and aquatic systems. Nitrogen is not a static entity, but takes part in a series of interconnected reactions that constitute the nitrogen cycle. During this cycle, part of the nitrogen is incorporated into biologically refractory organic material that is sequestered in soils and sediments from the overall nitrogen cycle. The significance of this refractory material to biological

productivity arises from the fact that much of its organic nitrogen resists further degradation by microorganisms and becomes sequestered from immediate use as growth factor for plants, microorganisms and aquatic organisms. Considering this, and the role of N in the overall nutrient cycle, it is of particular interest to understand the origin and the fate of N-containing organic compounds both during pedogenesis and in the sedimentary record.

The formation of refractory organic matter in soils and sediments is controlled by several factors, including the chemical composition and amount of material entering the system, the biological activity and the chemical and physical condition of the environment. Those factors, however, differ for soils and sediments due to the difference in developmental history, input material and differences in chemical and physical conditions. Thus, beside reviewing the present knowledge of N-stabilization during humification, the goal of the review is to elucidate the impact of different environmental factors on this process by discussing environments that differ in the origin of their organic matter precursors as well as in chemical and physical conditions. Those environments include recent and fossilized deposits formed from algal residues and vascular plant remains as well as mineral soils. In addition, the impact of vegetation fire on organic N composition in soils is discussed.

2. The global nitrogen cycle

On our planet, nitrogen (N) occurs in the atmosphere, the lithosphere and the biosphere. Overall, 98% of the nitrogen mass of the earth (197×10^{21} g) is contained in rocks and minerals (Porter, 1975). Approximately 3.8 to 3.9×10^{21} g nitrogen are estimated to occur in the atmosphere (Walker, 1977). The amount of nitrogen in the biosphere constitutes only 0.02% of the total global nitrogen mass of which 2.2×10^{16} g are estimated to be immobilized into living organisms (Porter, 1975). Most of the biosphere nitrogen, however, is found in soil organic matter (10.5×10^{16} g; Paul and Clark, 1996) and ocean-bottom organic nitrogen (5.4×10^{16} g; Porter, 1975).

The main part of the nitrogen available to the biota originally derives from nitrogen fixation either by lightning or by microbes. Values ranging from 1 to

100×10^{12} g year⁻¹ are estimated to originate from lightning. Most of this nitrogen is deposited in the sea (Liaw et al., 1990; Schlesinger, 1991). Newer estimates suggest a contribution of 3×10^{12} g year⁻¹ (Galloway et al., 1995). However, lightning is relatively unimportant as a reactive source compared to nitrogen fixation by bacteria. For total biological nitrogen fixation on land, a median value of approximately 140×10^{12} g N year⁻¹ is described in the literature (Burns and Hardy, 1975). The input of nitrogen by biological nitrogen fixation in the oceans of the world was estimated to be around 40 to 200×10^{12} g N year⁻¹ (Carpenter and Romans, 1991). Human contribution to nitrogen input in terrestrial systems via fertilizers produced by the Haber–Bosch process supplies approximately 80×10^{12} g N year⁻¹ (Schlesinger and Hartley, 1992; Jenkinson, 2001). Nitrogen fixed by combustion processes in automobile engines and industry, and released to the atmosphere constitutes about 40 to 60×10^{12} g N year⁻¹ (Rosswall, 1981; Warneck, 1988; Galloway et al., 1995), most of which precipitates over land.

Approximately 36×10^{12} g N year⁻¹ are carried from land to the sea by rivers, accounting for a large proportion of the nitrogen lost from land (Schlesinger, 1991). The remaining nitrogen that is removed from land is assumed to be lost by denitrification in terrestrial soils and wetlands and by N₂ released by forest fires (Lobert et al., 1990). Global estimates for denitrification in uplands and freshwater wetlands range from 13 to 233×10^{12} g N year⁻¹ (Bowden, 1986). Assuming a mean atomic carbon to nitrogen (C/N) ratio of 50 for plant biomass and a terrestrial net primary production (NPP) of 60×10^{15} g C year⁻¹, the nitrogen requirement of land plants is about 1.2×10^{15} g year⁻¹ (Schlesinger, 1991). Thus nitrogen fixation supplies approximately 10% to 20% of the nitrogen that is made available for plant use each year. The remaining nitrogen must derive from internal recycling and the decomposition of dead material in the soil. Plants are known to utilize only 30% to 60% of the available mineral nitrogen in soils. At an average efficiency of 40%, this nitrogen makes up approximately 3.5×10^{15} g or 3% of the soil nitrogen content (Paul and Clark, 1996). When the turnover time in the soil is calculated with respect to the input of dead plant materials, the mean residence time of nitrogen in soil organic matter is about 50 years (Schlesinger,

1991). In comparison, for the total pool of organic carbon in soils a mean residence time of slightly over 26 years was suggested (Schlesinger, 1991). However, evaluating these numbers one has to bear in mind that the mean residence time of organic material in soil varies over several orders of magnitude between the surface litter and the various humus. A further consideration that should be pointed out is that in particular for some N turnover times may be very long because of internal recycling of mineralized N.

Single-cell phytoplanktons in the photic zone of surface waters are the main contributors to NPP in marine systems. Assuming that NPP in the ocean fixes approximately 45×10^{15} g C year⁻¹ and an atomic C/N ratio of approximately 7 for phytoplankton, the latter must take up approximately 6.5×10^{15} g N year⁻¹ (Peterson, 1981; Schlesinger, 1991). Models show that most of the nitrogen for NPP in the surface waters is supplied by nutrient mineralization in the surface waters (Schlesinger, 1991). A part of the nutrients is lost to the deep ocean. Nutrients released during decay of biological material are largely recycled (immobilized) in the deep ocean, resulting in much higher nitrogen concentrations than observed in the upper water layers. Some of the free (NH₄⁺ and NO₃⁻) and immobilized (organic) nitrogen enters the sediments where further recycling occurs. A small pool of approximately 10.5×10^{12} g N year⁻¹ is buried and preserved in marine sediments (Schlesinger, 1991). Most of the input of nitrogen to the oceans, however, return to the atmosphere due to denitrification (150 to 180×10^{12} g N year⁻¹; Galloway et al., 1995).

3. Biological precursors of organic matter in soils and sediments

3.1. Aquatic systems

The primary producer in lakes and marine environments are mostly composed of diatoms, dinoflagellates, cyanophyceae (blue-green algae), chlorophytae (green algae) and very tiny phytoflagellates. Due to the absence of lignin and the low amount of other N-free structures, their average C/N ratio is near 6 (Redfield et al., 1963). They contain between 24% and 50% proteins, up to 40% carbohydrates and 2% to 10% lipids (Tissot and Welte,

1984). Some single-cell algae (i.e. *Scenedesmus*, *Botryococcus braunii*, *Tetradron minimum*) possess additional outer cell walls containing solvent insoluble macromolecules characterized by an unusually high resistance to chemical degradation and by a highly paraffinic structure (Berkaloff et al., 1983; Goth et al., 1988; Kadouri et al., 1988). Morphological and chemical comparison of these cell wall layers from fossil algal-derived sediments revealed strong similarity both in physical and chemical properties (Hatcher et al., 1983; Largeau et al., 1984).

Other important sources of accumulated organic material in aquatic environments include bacteria and zooplankton. Bacterial numbers in surface marine sediments (10^8 to 10^{10} cells g⁻¹ dry weight) resemble those in soils and decrease by about an order of magnitude downwards within the first 10 cm of these deposits (Deming and Baross, 1993). Copepods constitute the largest single fraction of zooplankton in most waters with 71% to 77% proteins, 5% to 19% lipids and 0% to 4% carbohydrates. In certain types of aquatic sediments, land-derived organic material, such as pollen, spores and other plant debris represent an additional allochthonous source.

3.2. Terrestrial systems

In terrestrial soils and bogs, most of the organic material derives from decaying vascular plant material. They are introduced to the soil by litter fall and roots. The roots are likely to contribute at least half of the organic matter input to soils (Schlesinger and Hartley, 1992). Vascular plant residues contain on average 15% to 60% cellulose and 10% to 30% hemicellulose (Haider, 1991). Lignin, a macropolymer of phenylpropane monomers, is an important compound of the secondary wall of woody cells in vascular plants and contributes approximately 5% to 30% of their total weight (Haider, 1991). Between 2% and 15% of the plant mass is assigned to N-containing compounds such as amino acids (Fig. 1), amino sugars (Fig. 2I), pyrimidines and purines (Fig. 3) or porphyrin structures (Fig. 4) (Haider, 1991). Besides these components, plant material contains lipids, waxes, resins, tannins and pigments (see review by Kögel-Knabner, 2002 and references therein). The relatively low content of N-containing compounds in most vascular plants results in their high C/N ratios,

neutral amino acids

acidic amino acids

basic amino acids

Fig. 1. Chemical structure of the natural amino acids.

i.e. 20 to 50 for tree leaves and 25 to 80 for herbaceous plants (Hedges et al., 1986; Lynch, 1988; Baldock et al., 1992).

In soils, microbial biomass comprises 2% to 3% of the total organic carbon in surface soil (Jenkinson and Ladd, 1981; Kassim et al., 1981). Microorganisms are concentrated in surface soils (Nelson et al., 1994) where they usually occur in a quiescent state unless activated by the presence of water and available substrates. Numerically, bacteria predominate over other types of microorganisms (Hassink et al., 1993). Although lower in number, fungi are larger than

bacteria, and thus, generally dominate (up to 70%) the total microbial biomass of the soil. Between 50% and 60% of the bacterial biomass can be assigned to N-containing compounds, most of which occur as proteins, peptides and amino acids. The relatively high content of N-containing biomolecules is responsible for the low C/N ratio of 5 to 8 of bacterial biomass (Paul and Clark, 1996). Bacteria exhibit lipid contents that are considerably higher than in plant material and comprise between 10% and 35% of the biomass (Swift et al., 1979). The content of water-soluble carbohydrates is between 5% and 30% with

Fig. 2. Chemical structure of (I) glucosamine and the repeating units of (II) peptidoglycan found in Gram negative bacteria and in (III) chitin.

Fig. 3. Structure of purines and pyrimidines found in nucleic acids.

cell walls containing between 4% and 32% (Paul and Clark, 1996). Bacterial polysaccharides primarily occur as lipopolysaccharides embedded in a lipid membrane and as amino sugar backbone of the peptidoglycan skeleton (murein) bound to cross-linking peptide chains (Fig. 2II).

Fungi contain approximately 14% to 52% N-containing compounds, 1% to 42% lipids and 8% to 60% water-soluble carbohydrates (Swift et al., 1979). Approximately 2% to 15% of the biomass can be assigned to cell wall polysaccharides (chitin) (Fig. 2III; Swift et al., 1979). Polysaccharides are found in the skeleton-forming inner and outer layers of the cell wall. The major contributor to the inner layer is chitin, an *N*-acetylglucosamine polymer associated with glucans or cellulose and embedded into various matrix polymers. The outer layer is composed of noncrystal-

Fig. 4. Basic unit of porphyrines (porphine) and chemical structure of chlorophyll a.

line polysaccharides (Peberdy, 1990). In some fungal cell walls, dark-colored pigments can be observed. The structure of these biopolymers, the melanins, is still a matter of debate (Saíz-Jiménez et al., 1995). Bell and Wheeler (1986) considered that some melanins originally identified as DOPA melanins are dihydroxynaphthalene melanins. Other studies indicated that the melanin fractions are complex mixtures of polysaccharides, proteins, lipids, nucleic acid derivatives and aromatic compounds (Saíz-Jiménez et al., 1995, Linhares et al., 1998, Coelho et al., 1997, Lüdemann et al., 1982) which may be linked together. Although occurring at low concentrations, these compounds are considered to provide protection against enzymes and solar irradiation (Bell and Wheeler, 1986; Butler and Day, 1998).

4. Analytical techniques for the characterization of organic nitrogen in soils and sediments and major findings

A typical procedure to isolate N-containing soil organic compounds, such as amino acids, amino sugars and nucleic acids, includes hydrolysis of the soil sample with 6 M HCl in a closed ampoule at a temperature of 100 to 120 °C for 12 to 24 h or under reflux. The hydrolysate is then purified and analyzed. After addition of ninhydrin to this solution, colored complexes are formed from the reaction of ninhydrin with the amino groups of amino acids and amino sugars. The amount of amino acids and amino sugars in the hydrolysate can be determined by measuring their concentration by photometric means. For the

identification and quantification of single amino acids, the hydrolysate is subjected to chromatography such as ion exchange chromatography, high performance liquid chromatography (HPLC) or gas chromatography (GC). Besides amino acids, the hydrolysate contains 1% to 2% amino sugars (Schnitzer, 1985) and traces of nucleic acids and other nitrogenous biomolecules such as chlorophyll, uric acid and phospholipidamines (Goodman and Cheshire, 1973; Cortez and Schnitzer, 1979; Schnitzer, 1985). Coelho et al. (1997) contributed about 4% to 5% of the total N in humic acids from six Brazilian topsoils and 0.5% to 1.5% of N in fungal melanins to amino sugars.

NH₃ produced during hydrolysis derives from ammonium and ammonia liberated after degradation of some amino acids, such as threonine or tryptophan, and/or nucleic acids. An accounting of all known potential sources of NH₃ in soil and sediment hydrolysates shows that about half of the NH₃-N, equivalent to 10% to 12% of the total organic-N, is still obscure (Schnitzer, 1985; Mayer et al., 1986). Some of this unknown hydrolyzable nitrogen was suggested to originate from pseudoamines, such as quinonimines, Schiff bases and enamines (Fig. 5), hydroxyamino acids, amino alcohols and sugars. Purines and pyrimidines (Fig. 3) were also suggested as a possible origin of this unidentified hydrolyzed nitrogen (Kickuth and Scheffer, 1976). After hydrolysis, a large portion of soil or sediment nitrogen, usually about one third, remains in the non-hydrolyzable residue. Because of its insolubility, this fraction is excluded from the investigation with common wet chemical approaches and is generally referred to as “unidentified nitrogen”. In the search

for the structure of this refractory organic nitrogen, most of the efforts were directed towards the identification of compounds which can resist microbial degradation and are inert against harsh chemical treatment. It was further concluded that such refractory organic nitrogen had to differ greatly from the labile nitrogen compounds of its biogenic precursors.

One possible method to study the structure of organic nitrogen that cannot be released by hydrolysis is analytical pyrolysis. This technique involves thermolytic degradation of macromolecules into small fragments that are analyzed by GC or gas chromatography–mass spectrometry (GC–MS). It is assumed that the fragments are representative of the original larger macromolecules. The interpretation of pyrolysis data, however, requires a detailed knowledge of the pyrolysis behavior of the compounds under study. Thermally induced secondary reaction can cause considerable modification of the original compound, which may bias the pyrolysis data. Thermal degradation of cellulose was shown to result in carbonyl compounds, acids, furanes, pyranones, anhydrosugars and phenols (Fig. 6I; Powels et al., 1989). Besides other compounds, the pyrolyzates of proteins include alkylpyrrolediones and pyrrolidinediones (Boon and de Leeuw, 1987; Fig 6II). Fatty acids may be decarboxylated under the pyrolysis procedure to form alkanes and alkenes. Nitriles found in soils and sediments could originate from the reaction of long-chain fatty acids and nitrogen derivatives during pyrolysis. Saíz-Jiménez (1994) suggested that the alkylbenzenes derive from artifacts during pyrolysis of triglycerides or unsaturated fatty acids in the presence of sulfur. The given examples illustrate the complexity of pyrolyzates obtained from macromolecular structures, and the difficulties involved in the interpretation of data obtained from pyrolytic studies of soil and sedimentary organic material.

Thermochemolysis with tetramethylammonium hydroxide (TMAH) avoids decarboxylation and produces methyl esters of carboxylic acids and methyl ethers of hydroxyl groups. This technique, originally introduced as pyrolysis with in situ methylation (Challinor, 1989), is used to convert polar products to less polar derivatives which are amenable to chromatographic separation (Challinor, 1989; Hatcher and Clifford, 1994; Saíz-Jiménez, 1994). It is possible to separate and detect many more structurally sig-

Fig. 5. Structure of some pseudoamines suggested to contribute to the unknown hydrolyzable N of soil organic matter.

Fig. 6. Examples of pyrolysis products from (I) carbohydrates (cellulose) and (II) proteins.

nificant products than observed previously by conventional pyrolysis–GC–MS. Recently, this technique was used to identify peptide structures in the hydrolysis residue of algal-derived recent and fossil sediments (Knicker et al., 2001; del Río et al., 2004). Schulten et al. (1997) used pyrolysis/methylation to identify uncharacterized soil nitrogen. They observed nitrogen derivatives of benzene and long-chain nitriles, not usually detected in pyrolysis–mass spectrometry of plants and microorganisms. They suggested that those compounds are characteristic of soils and result from stable transformation products of soil nitrogen. Such compounds were not detected after TMAH pyrolysis/methylation of red soybean protein powder and clover hay (Reeves and Francis, 1998).

Solid-state nuclear magnetic resonance (NMR) spectroscopy represents an alternative non-degradative technique, which allows complex heterogeneous mixtures to be examined without thermolytic or chemolytic pretreatment. Although it is difficult to obtain detailed compositional information, various general types of functional groups can be identified by the chemical shift values of certain resonance frequencies within a spectrum. Assuming that appropriate acquisition parameters are used, the determination of the signal intensities gives information about the average relative distribution of each identified functional group. While these advantages have made solid-state ^{13}C NMR spectroscopy a well established technique for the characterization of soil organic carbon (Wilson, 1987), the development of solid-state ^{15}N NMR of N-containing geopolymers has been hampered by the fact that its sensitivity is approximately 50 times lower than ^{13}C NMR. Consequently, ^{15}N NMR was mostly applied in

geoscience to studies involving ^{15}N -enriched material (Preston et al., 1982; Benzing-Purdie et al., 1986; Cheshire et al., 1990; Almendros et al., 1991; Thorn and Mikita, 1992; Zhuo and Wen, 1993; Knicker and Lüdemann, 1995; Clinton et al., 1996; Potthast et al., 1996; Bedrock et al., 1998; DiCosty et al., 2003). Although Preston et al. (1986) published a first solid-state ^{15}N NMR spectrum of humic material from a peat with natural ^{15}N -abundance and a nitrogen content of 4%, a routine application of this technique for the investigation of refractory organic nitrogen in soils and sediments only started recently after the systematic determination and optimization of important solid-state spectral parameters (Knicker and Lüdemann, 1995).

5. Models for the formation of recalcitrant organic nitrogen in soils and sediments

Natural organic matter in soils and sediments is defined as all organic components in and on the surface of these environments that derive from dead material of primary producers (plants and phytoplankton), animals and microbes. Most of this material becomes quickly mineralized into forms that reenter the nutrient cycle. A smaller part, however, is subjected to humification which involves degradation and/or synthesis, converting labile organic molecules into more recalcitrant organic forms of low microbial accessibility that can resist degradation for prolonged time periods both during soil development (pedogenesis) and sediment diagenesis. These processes may start before death, but are mainly the result of microbial reworking or chemical and physical processes occurring after cell death both before and after entering soils and sediment.

Several mechanisms are discussed to explain the formation of refractory organic matter in soils and sediments. The selective preservation pathway claims that refractory biopolymers resist biodegradation and accumulate while other more labile compounds are quickly mineralized during the early stages of humification in soils and in the water column and upper layer of sediments. This selective preservation pathway was first suggested by Philip and Calvin (1976), later by Hatcher et al. (1983) and more recently by numerous others (see review by de Leeuw

and Largeau, 1993). This hypothesis is supported by the finding that several vascular plants, algae, and bacteria produce hydrolysis-resistant biopolymers that are highly recalcitrant toward biodegradation. Further evidence supporting this pathway is obtained from comparative studies of such biopolymers extracted from the cuticles and cell walls of plants, and of the cell walls of microorganisms and a number of kerogens in ancient rocks (Largeau et al., 1984; Nip et al., 1987; Goth et al., 1988).

Other mechanisms involved in the protection of organic matter during humification are processes leading to a decrease of the microbial and enzymatical accessibility of formerly labile compounds. As suggested in the depolymerization–recondensation pathway, naturally occurring macromolecules are microbiologically degraded to oligomers and monomers, which for the most part are further mineralized. A small fraction of those compounds, however, recombine by random condensation to larger, insoluble and refractory structures which survive prolonged pedogenesis and sediment formation.

One of the early concepts recommending a depolymerization–recondensation pathway as a possible soil organic N stabilization mechanism suggests that humic material is formed by condensation of C=O groups of lignin with NH₂ groups of proteinaceous material to produce Schiff bases (Waksman and Iyer, 1932). Other models are based on the abiotic condensation of phenolic or quinonic structures with N-containing compounds. Possible mechanisms involve covalent binding of the ϵ -lysylamino groups of proteins on quinones formed during lignin degradation (Theis, 1945; Fig. 7I) or the condensation via H-bridges between the hydroxyl groups of phenols and the carboxylic groups of proteins (Ladd and Butler, 1975). Piper and Posner (1972) suggested the formation of *N*-methylcarboxyl-quinonimine complexes (Fig. 7II) or structures which resemble *N*-(*p*-hydroxyphenyl)-glycine and *N*-(*p*-hydroxyphenyl)-glutamine. The presence of acid-persistent organic nitrogen in soils was explained with acid-insoluble indoles formed as condensation products of quinones and/or phenols with amino acids (Rinderknecht and Jurd, 1958; Fig. 7III). Another widely accepted mechanism for the formation of recalcitrant nitrogen in organic matter of terrestrial systems is the formation of phenazine and

phenoxazone derivatives via 1,4 addition of ammonia or amino groups onto phenols or quinones and subsequent autopolymerization of these units (autopolymerization model; Flaig et al., 1975; Fig. 7IV/V). The phenols necessary for this reaction are thought to derive from the degradation of lignin or from secondary metabolites of fungi and microorganisms (Haider and Martin, 1967).

The concept of nitrogen stabilization via abiotic ammonia fixation on humic material has also been the subject of recent ¹⁵N NMR spectroscopic studies. Examining the structure of synthetic humic acids produced by air oxidation of benzoquinones with ammonium chloride, amino acids and peptides by means of ¹⁵N NMR spectroscopy, Preston et al. (1982) concluded that polymers are formed by coupling of semiquinone radicals. Amino compounds may be incorporated by formation of bonds between amino nitrogen and aromatic carbon. Zhuo and Wen (1993) on the other hand, assumed from their ¹⁵N NMR spectroscopic examination of synthetic humic acid that great differences exist between these synthetic compounds and natural humic acids. Investigating the reaction of ¹⁵N-labeled ammonium hydroxide with the Suwannee River fulvic acid, the IHHS peat and leonardite humic acids, Thorn and Mikita (1992) recovered most of the incorporated nitrogen in indoles, pyrroles, followed by pyridine, pyrazine, amide and aminohydroquinones (Fig. 8). Potthast et al. (1996), subjecting lignin model compounds to oxidative ammonolysis, identified the incorporated nitrogen mostly as amides and benzonitriles (Fig. 8). N-heterocyclic compounds were not found. They concluded that benzonitrile derivatives may also contribute to the active nitrogen-containing soil components that are released slowly from the soil. In aquatic systems, which in general are lignin depleted, formation of humic substances was suggested to occur via the Maillard reaction, during which carbonyl C reacts with amino groups (Maillard, 1916; Nissenbaum and Kaplan, 1972; Hedges, 1988; Ikan et al., 1996) to form dark colored melanoidins.

It has also been suggested that some fractions of soil and sedimental organic matter are preserved by interaction with the mineral phase. This may be due to intercalation between the silicate sheets of clay minerals (Theng et al., 1986) or incorporation into pores that are too small for entrance and functioning

Fig. 7. Reaction products suggested to be formed after abiotic condensation of phenolic or quinonic structures with N-containing compounds, according to Theis (1945) (I), Pipner and Posner (1972) (II), Rinderknecht and Jurd (1958) (III) and Flaig et al. (1975) (IV, V).

of degrading enzymes (Mayer, 1994a,b). Alternatively, organic matter in soils may be physically protected within micro- or macroaggregates (Tisdall and Oades, 1982). Such aggregates may be stabilized by biogenic secretions, polysaccharides or particles of plant and biomass residues (Haider, 1999; Tisdall et al., 1997). Golchin et al. (1994) proposed their origin

from microorganisms located on plant residues and sorbed to mineral particles which encapsulate the biogenic remains and thus protect them from further degradation. Wershaw (1999) suggested that aggregates can be formed by humic amphiphiles in the leachate from the decomposing litter layer that percolates through a soil column. They interact with

Fig. 8. Products detected by ^{15}N NMR after reaction of ^{15}N -labeled ammonium hydroxide with humic material (Thorn and Mikita, 1992) and after oxidative ammonolysis of lignin model compounds (Potthast et al., 1996).

the soil mineral grains to form bilayer membrane-like coatings on hydrous oxide and clay minerals.

6. Degradability of organic N compounds

Most of the organic nitrogen in soils and sediments ultimately derives from living organisms where it is mainly present as peptides, amino acids and to a lower extent as amino acids. In Fig. 9, the solid-state cross polarization magic angle spinning (CPMAS) ^{15}N NMR spectrum of the biomass from *B. braunii* is shown. Comparable spectra were obtained for other algae (Derenne et al., 1993; Knicker, 2000a), plant residues (Knicker and Lüdemann, 1995; DiCosty et al., 2003) and fungal biomass (Bedrock et al., 1998). In all those spectra, the predominance of peptide N is confirmed by the intense signal around -260 ppm and a smaller signal around -346 ppm. The latter is assigned to terminal amino groups in peptides, N in amino sugars or the ϵ -N in lysine (Table 1). Further components, which occur in lower amounts in biomass, are nucleic acids, alkaloids and porphyrines. Their N contributes to the shoulder between -210 and -245 ppm.

Most of these biomolecules, however, are expected to be quickly mineralized. In most surface environments, they are not expected to survive more than 10^5 years (Bada, 1998). In model experiments, it was shown that after adding ^{14}C -labeled glycine to a Mollic Haploxeralf, 75% and 89% of their labeled carbons was released after 1 and 12 weeks of incubation, respectively (Verma et al., 1975). From

added tripeptide glycyl-glycyl-leucine, approximately 80% of the labeled carbon was converted into CO_2 within 4 weeks (Verma et al., 1975) and about 73% of the ^{14}C was released from added glucosamine after 12 weeks of incubation (Bondietti et al., 1972). Adding caffeic acid or cysteine to a sandy loam, up to 67% and 75% of the substrate was mineralized after 12 weeks (Martin and Haider, 1986).

Incubation of some non-melaninic fungi, streptomycete, and algal cells in Greenfield sandy loam soil revealed degradation rates comparable to that of wheat straw (Kassim et al., 1981). In all experiments, approximately 60% of the added carbon was lost within 12 weeks (22°C). Comparable experiments with young leaves of *Lolium perenne* incubated with quartz sand at 25°C and a sample moisture of 60% of the maximal water holding capacity showed a C-loss of 80% after 2 years of incubation (Knicker and Lüdemann, 1995). Water saturation conditions only slightly decreased the C-loss to 75% (Knicker et al., 1996a). Solid-state ^{13}C NMR experiments indicated that this loss affected all C groups, although the relative contribution of the carbohydrate fraction decreased the most. However, analysis of the degrading plant residues by solid-state CPMAS ^{15}N NMR showed that in spite of an N-loss of 80% by volatilization, almost all of the remaining organic N was bound in proteinaceous structures, part of which was even resistant against drastic acid hydrolysis. Formation or a relative enrichment of heterocyclic compounds or pyrrole structures during humification was not observed. Those results demonstrated that in spite of the high lability of proteins and amino acids, plant material contains proteinaceous material which is protected against biological and chemical degradation, possibly by steric hindrance (Almendros et al., 1991) or by physical protection through refractory biopolymer (Knicker et al., 2001).

Resistance of amides against microbial degradation was also observed for a mixed algal culture (*Chlamydomonas*, *Chlorella*, *Closteridium*, *Scenedesmus*) incubated for 2 months under aerobic conditions (Knicker et al., 1996b). Two-dimensional solid-state ^{15}N ^{13}C NMR spectroscopy with which the N-functionality of the ^{15}N NMR spectrum can be correlated to the directly bound ^{13}C group confirmed the assignment to peptide structures (Knicker, 2000b). Calculations indicated that about 45% of the total C of

Fig. 9. Solid-state ^{15}N NMR spectrum of *B. braunii* and the solid-state ^{13}C and ^{15}N NMR spectra of the algaenans *B. braunii* and *S. communis*, the rubbery remains of *B. braunii* deposited on lake Balkash (Balkashite) and derived from the Coorong area (Coorongite).

Table 1
Assignments for the various chemical shifts in a ^{15}N NMR spectrum

	Assignment to biological material	Other assignments
25 to -25 ppm		Nitrates, nitrites, nitro groups
-25 to -90 ppm		Imines, phenazines, pyridines, Schiff bases
-90 to -145 ppm	Purine (N-7)	Nitrile groups
-145 to -220 ppm	Chlorophyll, purines/ pyrimidines, indoles, imidazoles, pyrroles,	Maillard products
-220 to -285 ppm	Amides/peptides, N-acetyl derivatives of amino sugars, tryptophanes, prolines	Lactame (pyrroles, carbazoles, indoles)
-285 to -325 ppm	NH in guanidines	Aniline derivatives
-325 to -375 ppm	NH_4^+ , NH_3^+ , NH_2 , NHR and NR_2 groups, free amino groups in amino acids and amino sugars	Anilinium salts

the algal residue after 2 months of incubation is assignable to proteinaceous compounds (Knicker, 2000a).

The high percentage of peptide-like material in the algal remains after 2 months of incubation indicates that some proteinaceous material was able to survive microbial digestion. It may derive from algal remains that persist against microbial attack but could also originate from resynthesis of microbial biomass. It seems very likely that such material could also survive the passage through the water column to serve as a major precursor of sedimental organic nitrogen. This assumption is supported by recent CPMAS ^{15}N NMR studies on dissolved organic matter from the Gulf of Mexico (McCarthy et al., 1997). These spectra, too, exhibit their main intensity in the chemical shift region assigned to amides and show no major intensity assignable to heteroaromatic-N. It appears that the formation of such compounds does not represent a major stabilization mechanism of organic nitrogen during its passage through the water column.

A typical refractory component in algae is algaenan, a highly paraffinic macromolecule. Fig. 9 depicts the solid-state ^{13}C NMR spectra of the algaenans from *Scenedesmus communis* and *B. braunii*. The highly paraffinic structure is indicated

by the dominating signal at 30 ppm (alkyl C). Olefines add to the small signal at 130 ppm and some ethers cause the shoulder at 73 ppm. Aldehyde C and carboxyl/amide C are responsible for the intensity at 208 and 172 ppm. This biopolymer occurs in the outer cell wall of some microalgae and was shown to be selectively preserved during formation of recent and fossil sediments (Hatcher et al., 1983; Largeau et al., 1984; Goth et al., 1988). Analysis of this biopolymer from *S. communis* (Derenne et al., 1993, 1997), and *B. braunii* (Fig. 9) by solid-state ^{15}N NMR showed signals assignable to amide N (-260 ppm) and free amino groups (-330 to -350 ppm). Subjecting the algaenan of *S. communis* to thermochemolysis with tetramethylammonium hydroxide confirmed the presence of peptides (Knicker et al., 2001).

Algaenan also represents the main component of Coorongite (Fig. 9) from the Coorong area in South Australia (Cane, 1969) and Balkashite from a deposit located around Lake Balkash, Kazakhstan (Gatellier et al., 1993). These materials were formed when the floating biomass of *B. braunii* resulting from extensive blooms was pushed by the wind onto the lakeshores. The resulting accumulations, upon drying, air and light exposure was transformed into a rubbery material (Cane, 1969; Wake and Hillen, 1980; Gatellier et al., 1993). Comparable to the algaenans, their solid-state ^{15}N NMR spectra show a strong signal around -260 ppm (Fig. 9). This signal confirms that some amides exhibit a very high resistance against early diagenetic reworking under the harsh conditions leading to the formation of such deposits. Their survival was recently explained with the encapsulation pathway (Knicker and Hatcher, 1997, 2001). According to this model (Fig. 10), it is possible that the amides may derive from proteinaceous material covalently bound to the recalcitrant biopolymers. During degradation, it becomes surrounded by the refractory structures and thus encapsulated in their network while other, more accessible compounds, are digested by microorganisms (Junction Pathway). It is also likely that the protected proteins comprise parts of the algal cell walls and that they become sandwiched between algaenan layers during microbial degradation and further diagenesis (Sandwich Pathway). Due to the hydrophobic nature of the algaenan, hydrophilic enzymes will not have access to certain key structures of the proteinaceous

Fig. 10. Conceptual scheme for the preservation of proteinaceous material in algal-derived organic remains (modified after Knicker and Hatcher, 2001).

material which, therefore, resist enzymatic degradation. Only conditions that alter the steric configuration of the paraffinic macromolecule may liberate the proteinaceous compounds such that they become accessible to microbial activity and chemical extraction. Encapsulation of labile protein into refractory organic matter may not be restricted to algal material but could also be associated with other hydrophobic or protective biopolymers such as cell wall components of bacteria or waxes or lignin in plant material.

In soils, fungal melanins were discussed as a possible precursor for refractory nitrogen. They have an important function in protecting the fungal cells from microbial degradation. Such cells (*Stachybotrys chartarum* and *Hendersonula toruloidea*) were shown to be more resistant to microbial decay than fungal cells without melanins. Less than 37% of the cell wall carbon of melanin-containing cells was released within 12 weeks of incubation at 22 °C. Removing the melanins by extraction and incubating the remains almost doubled the carbon loss (Kassim et al., 1981). Degradation of the isolated melanins (*H. toruloidea*, *Aspergillus glaucus*, *Eurotium echinulatum*) showed that less than 10% of their carbon evolved as CO₂ (Linhares and Martin, 1978). In this study, these

fungal polymers were almost as resistant to biodegradation as soil or peat humic acids. Zavgorodnyaya et al. (2002) report a C-loss of 22% and 25% from the melanins of *Aspergillus niger* and *Cladosporium cladosporioides* after 3 months of incubation. Those authors, however, found that under the used laboratory conditions both fungal melanins were more readily degradable, had more functional groups and relatively higher molecular weight than humic acids from soils and brown coal. The changes in elemental composition, optical parameters and the decrease of molecular weight during degradation, on the other hand, made them more similar to soil humic acids.

Linhares and Martin (1978) suggested that melanin-forming fungi constitute a major fraction of the soil fungal biomass and that it is likely that these polymers contribute to the resistant humus. On the other hand, comparisons of the melanin from *H. toruloidea* indicated that the melanin has less carbon and sulfur but more nitrogen and oxygen than humic acids (Schnitzer and Chan, 1986). Additionally, solution ¹³C NMR spectra from various fungal melanins showed fewer similarities to humic acids than would be expected from the analytical data in the literature (González-Vila et al., 1978; Lüdemann et al.,

1982). Lüdemann et al. (1982) conceded that in ^{13}C NMR spectra of humic acids, the specific ^{13}C -signals of the melanins and their altered remains could be masked by the overall signals of the complex mixture of humic acids. It was further proposed that although melanins are very resistant to microbial degradation, certain parts of the melanin may degrade faster than others and with time, the melanin-derived units would become more like aged soil humic material. Concerning their nitrogen functionality, it was observed that the melanin of *H. toruloidea* contained more nitrogen that resisted hot acid hydrolysis than did the humic acids (Schnitzer and Chan, 1986). It was assumed that this nitrogen is bound in heteroaromatic polymers whose high occurrence in the melanin was taken as an indication that fungi may be able to produce them from relatively simple starting materials. However, recent solid-state ^{13}C and ^{15}N NMR spectroscopic studies of melanins from different fungi (*E. echinulatum*, *Trichoderma harzianum*, *Ulocladium atrum*, *H. toruloidea*, *Oidiodendron tenuissimum*) demonstrated, on the one hand, large differences in the chemical composition of melanins from different species, thus, showing a high variability of this compound classes (Knicker et al., 1995). For all of them, more than 70% of their nitrogen was assignable to amide-N and even under the most favorable conditions, heteroaromatic-N could not contribute more than 15% to total N-intensity. This indicates that their structure is significantly different from those melanins produced in synthetic dopa- and natural melanoma- and sepia-melanin (Duff et al., 1988). However, if fungal melanins form a considerable fraction of the refractory organic matter in soils, those structures with their high amide N content will contribute to the dominance of peptide structures in humic material, rather than being a possible precursor for the heteroaromatic-N fraction.

7. Stabilization by depolymerization–recondensation

In contrast to laboratory studies, giving evidence that the depolymerization–recondensation pathway could be possible, neither N-heterocyclic compounds, nor benzonitrile were identified in solid-state ^{15}N NMR studies in which natural lignin, beech sawdust,

Rye grass (*L. perenne*) and wheat straw were subjected to microbial degradation after addition of $(^{15}\text{NH}_4)_2\text{SO}_4$ for 600 days (Knicker et al., 1997). Almost all of the organic nitrogen was assignable to peptide-like structures. This was confirmed by 2D solid-state ^{15}N ^{13}C NMR measurements (Knicker, 2002) and suggest that during humification, inorganic N is immobilized and stabilized into microbial biomass within a couple of weeks. After acid hydrolysis of the rye grass residue, incubated for 2 months, only 7% of the N was recovered in the hydrolysis residue (Knicker, 2003). This may indicate the lower recalcitrance of microbial organic N in comparison to organic N in plant and algal biomass.

On the other hand, those studies may not necessarily prove that recondensation of N-organic compounds with humic material does not occur in soils and sediments. Indications for such reactions derive from studies, examining the humification of the explosive 2,4,6 trinitrotoluene (TNT) in soils (Knicker, 2003). Incubating ^{15}N -TNT with ^{13}C -enriched plant material both under aerobic and anaerobic conditions revealed covalent binding of the reduced amino groups of TNT on the ^{13}C -enriched organic matter. 2D solid-state ^{15}N ^{13}C NMR identified amides as the binding functional group. Different to the amides detected in the plant residues incubated with $(^{15}\text{NH}_4)_2\text{SO}_4$, those formed by transformation of TNT revealed a high resistance against acid hydrolysis. Thus, the condensation products are unlikely to be formed by incorporation of ammonium or nitro groups released during TNT degradation into microbial biomass. The amide formation during humification of TNT is more likely to have been mediated by acetylation/formylation and alkylation of amines produced by the ongoing reduction of TNT-transformation products (Knicker, 2003; Fig. 11). Such reactions were reported to occur during biotransformation of TNT by *Phanerochaete chrysosporium* (Hawari et al., 1999) but also during TNT transformation in non-sterilized soils (Sheremata et al., 1999). Those reactions may serve as a detoxification mechanism. Likewise, those amides may have been formed by condensation reactions with the carboxylate groups of microbial cellular components as it was suggested by Carpenter et al. (1978).

In particular in terrestrial ecosystems, the formation of protein–tannin complexes is a further mech-

Fig. 11. Conceptual scheme for the immobilization of the explosive 2,4,6-trinitrotoluene into soil organic matter according to Knicker (2003).

anism assumed to be responsible for the stabilization of proteins. Tannins are secondary plant metabolites occurring in the bark and foliage of most plant species. In woody species, foliar tannin levels range between 15% and 25% dry weight, in roots between 1% and 35% are reported (Kraus et al., 2003; and references therein). Tannins are built up by flavan-3-ol units (condensed tannins) or gallic acid monomers (hydrolyzable tannins). Protein–tannin complexes can be formed in the soil during litter decomposition but also already during leaf senescence (Kuiters, 1990) and are expected to resist degradation by most microorganisms. Some ectomycorrhizal in coniferous forest soils, however, are able to mineralize such compounds. Recent studies, examining the chemical alteration of condensed tannins of pine needles and willow leaves during litter degradation by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF) and solution ^{13}C NMR spectroscopy (Behrens et al., 2003; Maie et al., 2003) could not confirm the high refractory nature of condensed tannins. Already after 8 weeks of incubation, no signal of condensed tannins was detectable by MALDI-TOF MS. Those studies indicated a continuous decrease of polymer chain lengths with an increase of carboxyl C on the expense of phenolic groups. Those changes correlated with the decrease of the protein binding ability of the tannins. In search of the nature of the binding between protein and condensed tannins, ^{15}N -enriched rye grass residues were incubated with isolated condensed tannins and

characterized by solid-state ^{15}N NMR spectroscopy. As shown in Fig. 12 by the dominating amide signal at -260 ppm and the missing of considerable intensity in other regions, stabilization of organic N did not occur via formation of heterocyclic aromatic

Fig. 12. Solid-state ^{15}N NMR spectra of ^{15}N -enriched plant residues (*L. perenne*) which were microbially degraded after addition of condensed tannins from spruce for 2 and 4 weeks. Asterisks indicate spinning side bands.

polymers. According to this observation, it is more likely that stabilization of the protein–tannin complex occurs via hydrophobic or van der Waals interactions. However, as indicated from the studies of TNT-humification, condensation by formation of amide bonds, at the present stage of research, cannot be excluded.

8. Organic-matter-rich sediments

Organic-matter-rich sediments refer to deposits with more than 1% organic carbon and are usually restricted to environments in which organic matter is produced faster than it can be degraded by micro-organisms or removed by an agent of transportation such as water and wind. Particles are allowed to settle and overlay older and more matured layers. The limited flux of material within a layer and throughout different layers in organic-matter-rich sediment often allows the possibility to relate the depths of the deposited layers to certain stages of development. Although chemical and physical conditions alter and anaerobic processes replace aerobic processes with increasing sediment depths, those conditions remain fairly constant within a layer. Consequently, organic-matter-rich sediments represent a fairly closed, homogeneous and persistent environment in which changes in chemical and physical properties of sedimentary material as a function of depth can reflect major alterations that have occurred during its maturation. Examples of environments with the potential of organic-matter-rich sediment formation are peat bogs, lake beds, the river deltas of continental shelves, and upwelling areas of marine systems. Other typical areas of high organic matter deposition are marine basins with draining waters less concentrated in salt than the incoming water. In general, the latter is nutrient-rich but oxygen-poor, supporting the formation of reducing conditions. A well known example of such a basin is the Black Sea which is considered a model for the preservation of organic-matter-rich sediments with a potential for oil formation.

During diagenesis, biochemical reactions are mainly responsible for organic matter transformation. At this time, the influence of temperature and pressure are limited. Aerobic microorganisms are restricted to the uppermost layer of sediments. In oxygen-depleted

layers, anaerobes reduce sulfates. While labile biomolecules are degraded, more stable primary compounds accumulate together with secondary products to form geopolymers which are precursors of kerogen in marine or deep lacustrine sediments and lignite in coal bogs. Continuing diagenesis leads to consolidation of the accumulated material, reduction in water content and an increase in temperature, due to increase in pressure. Microbial activity decreases and finally stops at a later stage referred to as catagenesis. In addition, organic–inorganic interactions attribute to organic matter stabilization at all stages of sediment evolution.

8.1. Algal-derived deposits

The chemical structure of N-containing compounds in fossilized algal deposits and the diagenetic mechanism implicated in their formation are far from established. [Patience et al. \(1992\)](#), examining samples from the Peru upwelling area by pyrolysis and X-ray photoelectron spectroscopy, identified four different nitrogen functions, namely pyridine, pyrrole, amides and, tentatively, quaternary nitrogen. The heterocyclic structures (pyrrole and pyridine) were predominant with pyrroles more abundant than pyridines. For the shallowest sample in the core (early Holocene), the amide-N represented at most 40% of the total nitrogen. Moreover, the relative content of amides was shown to decline with increasing sample depth. It was suggested that the changes in N-functionality began at or above the sediment/water interface. Those results are in contrast to recent solid-state ^{15}N NMR studies on a Holocene sapropel from Mangrove Lake, Bermuda ([Knicker and Hatcher, 2001](#)). In this environment, the organic matter is not diluted extensively by mineral matter (mineral content <10%) as it is typically found in marine sediments and most soils. Stabilization of N via adsorption onto minerals or entrapment into mesopores that are too small for enzyme access ([Mayer, 1994a,b](#)) thus will not represent the dominating preservation pathway.

The solid-state ^{15}N NMR spectroscopic studies showed that, comparable to the degradation studies of fresh algae, the nitrogen in all fractions of all depths is bound mostly in amides ([Knicker and Hatcher, 2001](#)). No indication of the formation of heteroaromatic-N with increasing organic matter maturation was

obtained. Based on earlier findings that algaenan dominates the chemical composition of the older layers of the bulk sapropel (Hatcher et al., 1982) and the fact that here, most of the nitrogen was associated with the humin fraction and partly survived acid hydrolysis, the survival of proteinaceous materials was explained through interaction with refractory algaenan via the encapsulation pathway (Knicker and Hatcher, 1997, 2001; Fig. 10). However, considering anaerobic conditions and the high sulfur content of the sapropel, sulfur-bridging, as a kind of natural vulcanization, may also be involved in the stabilization of N-containing aliphatic chains. Recently, Riboulleau et al. (2002) suggested that encapsulation into aliphatic sulfurized material was probably the

major process for amino acid preservation in high sulfur content kerogens.

Nevertheless, although proteinaceous material was identified in kerogen after the release of amino acids upon tetramethylammonium hydroxide (TMAH) treatment of the polar fraction of a 140 million years old kerogen (Mongenot et al., 2001; Riboulleau et al., 2002), solid-state ^{15}N NMR spectroscopy confirmed that the dominance of amides does not survive into the fossil stage (Knicker et al., 1996a,b; Derenne et al., 1997). As shown in Fig. 13, the main signal in the solid-state ^{15}N NMR spectra of algal-derived fossilized sediments is shifted towards lower fields and embraces the region assignable to pyrrole and indole structures (-170 to -250 ppm). However, the upfield

Fig. 13. Solid-state ^{13}C and ^{15}N NMR spectra of algal-derived fossil sediments.

shoulder still may contain some intensity derived from amide structures. Dominance of pyrrole N was also found in the solid-state CPMAS ^{15}N NMR spectrum of Green River kerogen (Knicker et al., 1996b). A smaller signal was identified in the region of pyridinic N. The presence of those two N-structures were recently confirmed by Kelemen et al. (2002) using XPS and solid-state CPMAs ^{15}N NMR. Their comparative study demonstrated good agreement between XPS and ^{15}N NMR data, indicating that if hetero-aromatic N is present in higher amounts it should be detectable by solid state ^{15}N NMR.

Because the marked change in nitrogen functionality from amides in recent algal-derived deposits towards pyrrolic components is observed for both the kerogens related to *Scenedesmus* and in those derived from *B. braunii*, this may indicate a common pathway for nitrogen transformation during fossilization. Such a change in the nitrogen functions takes place at an earlier stage than observed for the carbon function, as indicated by the relatively constant intensity distribution among the solid-state ^{13}C NMR spectra of the immature sediments. They reflect the dominance of paraffinic structures which is in accordance with the selective enrichment of algaenans during sediment formation. Only the mature Torbanite shows a considerable increase in aromaticity. This observation may allow the assumption that organic nitrogen follows a maturation pathway independent from that of the carbon skeleton of the algaenan. The differing behavior of the carbon and nitrogen fraction points to the assumption that the nitrogen surviving prolonged sediment formation is chemically separated from the aliphatic network of the algaenan, rather than being an intrinsic part of, or tightly bound to this refractory structure. A possible explanation for the change in nitrogen functionality during fossilization may be the selective elimination of amides and the enrichment of preexisting indole- and pyrrole-containing biopolymers (Derenne et al., 1997). The identification of porphyrins in aged sediments strongly supports such a pathway. Alternatively, proteinaceous material that has survived early diagenesis may undergo abiotic diagenetic rearrangements during burial. Hydrolysis could then liberate ammonia that may interact with reactive functional groups in other organic compounds and polymerize to form heterocyclic-N polymers. Such polymers could also be

formed via cyclization of amide-containing aliphatic chains or peptide structures, as observed in pyrolytic studies of proteins (Boon and de Leeuw, 1987). Further studies are needed to discriminate whether heterocyclic nitrogen in kerogens originates from preexisting diagenetic products and/or are diagenetically generated.

8.2. Deposits formed with input of vascular plant remains

In terrestrial environments, most of the deposited organic material derives from plant remains and bacterial input. In contrast to algae, plant material contains lignin that is considered as a major precursor of stabilized organic matter in peat and coals. Like sapropels, peats develop in relatively homogeneous environments with low inter- and intra-spatial fluxes. Thus, they represent, comparable to sapropels, systems in which sediment depth can be related to the extent of diagenetic alteration. As peat develops where the water table is close to the soil surface, aerobic processes are replaced by anaerobic processes with increasing depth. In general, activity is high at the surface of peats but decreases rapidly within the first few centimeters of depth. With increasing depth, anaerobic bacterial activity dominates over fungal and aerobic bacterial activities.

In peats, anaerobic decomposition of plant material is selective. Analyzing peatified stem wood of *Calluna vulgaris* (Scotch heather; van der Heijden and Boon, 1994) and various other peatified vascular plant remains at different stages of peatification (Bates et al., 1991; Dudley et al., 1990), a preferential decrease of carbohydrates, such as cellulose and hemicellulose, relative to lignin was observed. Beside plant remains, fungal and microbial residues are contributing to the organic matter in peat (Dudley et al., 1990). The identification of sterols of algal origin in some Spanish peats and lignites (del Río et al., 1992) indicates that algal residues may also have an impact on its organic composition.

The analysis of the N-fraction of a peat from Torreblanca, Spain, by solid-state ^{15}N NMR spectroscopy (Knicker et al., 2002) demonstrated that comparable to the recent algal deposits, almost all of the organic nitrogen occurs in peptide-like structures. The same result was also obtained from the analysis of

Fig. 14. Solid-state ^{15}N NMR spectra of the peat layers below the sapropel from Mangrove Lake, Bermuda (modified after Knicker et al., 2002).

a peat layer (Fig. 14), directly underlying the sapropel from Mangrove Lake. Subjecting a peat humic and fulvic acid (standards from the International Humic Substance Society) to nitrogen K-edge XANES spectroscopy supported the dominance of peptide N in such samples, although some intensity was assigned to heterocyclic N (Vairavamurthy and Wang, 2002). Considering that the mineral content of most peats is too low to account for considerable N-preservation, physical protection of potentially labile

organic N compounds within nondegradable organic structures by the encapsulation pathway may also be responsible for N-stabilization in bogs. However, in the layer at 16.2 m of the Mangrove Lake peat, a broad shoulder assignable to pyrrole N was detected (Fig. 4). In the solid-state ^{15}N NMR spectrum of lignite from the Arenas del Rey deposit (Spain) (Knicker et al., 2002) and several bituminous coals (Knicker et al., 1996a), this signal dominates comparable to the fossilized algal deposits. These results indicate that the formation of heterocyclic N is a common maturation pathway of organic N during fossilization and may start during the boundary between diagenesis and catagenesis. However, pyridine commonly assumed to be a major N-component of coals was up to now only detected in the solid-state ^{15}N NMR spectrum of coalified wood at the maturation stage of anthracite (Fig. 15) giving rise to a signal at -82 ppm.

9. Organic nitrogen in mineral soils

Soils develop where rocks, rainwater, atmospheric gases and living organisms meet and interact. Soils can be described as natural systems in which parent rock material alters under certain climate, topography, and due to the input of remains of a certain vegetation. Such pedogenic processes include weathering of rocks

Fig. 15. Solid-state ^{13}C and ^{15}N NMR spectra of a high volatile A bituminous coal and coalified wood (modified after Knicker et al., 2002).

and minerals, degradation and humification of organic material, alteration of the texture, but also translocation and transformation of soil components. Pedogenesis is a continuous process occurring over an extended time period with continuous production of soil material. The quantity and quality of formed soil is controlled by the conditions of the environment, resulting in a high variability of soil types. The production of soil material is opposed by losses due to erosion and leaching of soluble inorganic and organic compounds into rivers and finally transport to the ocean. It is important to note that soil is not a static entity but can be regarded as a transient environment with continuous exchange of materials and energy across its boundaries and within its matrix (Hedges and Oades, 1997).

Various soils are characterized by horizons with specific properties, and reflecting the individual pedogenetic history and processes which have occurred during pedogenesis. The deepest horizon (C horizon) is composed of partially disintegrated and decomposed rock material of unconsolidated sediments as well as of solid bedrocks. Above this horizon, the B horizon represents a zone where weathering has proceeded further and only those minerals of the parent rock which are most resistant to decomposition are recognizable. The other minerals have been converted into new minerals and into soluble salts which can leach. This material can be mixed with humified organic material, clay minerals, iron oxides and calcite deposited after percolating from the overlying A horizon. The A horizon is enriched by organic material due to leaching of colloids and soluble materials from the overlying litter layer, the O horizon, which primarily consists of partially degraded plant residues.

Compared to organic-matter-rich deposits, mineral soils show a much higher heterogeneity with respect to material and energy fluxes within and across their boundaries. Also, within soils, differences in pore sizes create microenvironments that differ in available space for microorganisms and supply in water, oxygen and nutrients. All these factors lead to a great spatial and temporal variability of the conditions that affect organic matter degradation. Consequently, soil organic matter can be considered a mixture of simultaneously occurring fractions at different stages of humification which have been formed both under

aerobic and anaerobic conditions. This heterogeneity is increased by bioturbation caused by earthworms and soil animals.

In spite of the differing conditions in soils and organic-matter-rich sediments, some comparable trends in refractory organic matter formation were observed in different soils at least in the above ground humic layer and the A horizon. (Zech et al., 1992; Kögel-Knabner, 1993) They are depicted in a decline of the C/N ratio and a considerable relative decrease of intensity in the *O*-alkyl C region and an increase of intensity in the alkyl C, aryl C and carboxyl C regions relative to the values for the biogenic precursors. Determination of the radiocarbon age revealed further an increase with soil depth (Scharpenseel et al., 1989; Rumpel et al., 2002). Based on solid-state ¹⁵N NMR spectroscopic studies on bulk soils that developed without having experienced fire (Knicker et al., 1993; Knicker, 2000a), it can be concluded that as in recent organic-matter-rich sediments, most of the organic nitrogen is immobilized in peptide-like materials and that heteroaromatic-N occurs only in minor amounts (Knicker et al., 1993). The dominance of amide-N (67% to 90% of total N) was also detected for humic acids extracted from some Japanese soils by means of XPS (Abe and Watanabe, 2004) and for a IHSS humic acid from a soil subjected to XANES (Vairavamurthy and Wang, 2002). Since microbial biomass comprises 2% to 3% of the total organic carbon in surface soil, one possible explanation for this could be incorporation of nitrogen into peptides of newly formed microbial biomass from nitrogen released during the breakdown of decaying organic material. Subjecting a Luvisol (Siebert et al., 1998) to hot acid hydrolysis with subsequent CPMAS ¹⁵N NMR spectroscopic examination of the extraction residue, however, revealed that as with the sapropel from Mangrove Lake, at least some of the amides and aliphatic amines survive this harsh treatment. Applying TMAH/thermochemolysis to this HCl-hydrolysis residue, some of the same products that were also obtained from albumin, were identified (Siebert et al., 1998). This reveals that also in soils some peptides exist that survive harsh chemical treatment, possibly by protection within other refractory biopolymers or as part of remains of fungal melanin.

As mentioned above, soils are a mixture of mineral and organic matter. It was shown that refractory

organic matter in soils is intimately associated with the silt and clay fractions (Baldock et al., 1992; Christensen, 1992), although some young organic material is present in these fractions (Baladane, 1996; Christensen, 1996). Solid-state ^{15}N NMR confirmed that the organic N in those fractions is also mainly assignable to proteinaceous material (Knicker et al., 2000; DiCosty et al., 2003). Peptide C contributed 8% to 25% to the total carbon of the fractions. Long chain aliphatic C was not detected to an amount that could explain peptide protection solely by the encapsulation mechanism. Here, additional protection may occur via chemical stabilization by chemical interaction, i.e. adsorption onto the surface of clay minerals or metal sites. Another mechanism involves complexing with polyvalent cations (e.g. Fe^{3+} or Al^{3+} ; Sollins et al., 1996) or entrapment into micropores (Mayer, 1994a). Leinweber and Schulten (2000) concluded that binding to pedogenic oxides contribute largely to the stabilization and non-hydrolyzability of peptides in soils. However, at the present stage of research, it is not possible to discriminate between or to favor one of the discussed mechanisms that may be responsible for the protection of peptide-like structures in mineral soils. It seems likely that they occur concomitantly. Their impact on soil organic nitrogen stabilization, thus, may be dependent upon the respective chemical and physical conditions of the environment.

Organic matter in the mineral horizons derives from root residues and exudates and from remains of the above ground litter that was transported to the deeper horizons by action of animals but also by leaching and precipitation of water-soluble and colloidal organic matter. It was suggested that about 10% to 25% of the total C input to forest floor with litter fall is leached from the organic surface layers (Guggenberger and Zech, 1992). About 60% to 90% of the leached dissolved organic carbon was found to become retained by the subsoil horizons within 15 min (Kaiser and Zech, 1998), possibly by sorption onto metal ions such as Ca^{2+} , Al^{3+} and $\text{Fe}^{2+/3+}$ (Guggenberger and Kaiser, 2003). Negatively charged compounds may be bound to the clay surface by cation bridges (Theng et al., 1986). Those binding mechanisms are likely to contribute to the stabilization of dissolved organic matter in subsoils (Guggenberger and Kaiser, 2003) and may be responsible for the strong resemblance of the chemical composition of organic matter in subsoil with the

Fig. 16. Solid-state ^{15}N NMR spectra of the A and AB horizons of a Rhodic Ferralsol from Brazil (modified after Dick et al., 2004).

hydrophobic dissolved organic matter from the overlying forest floors (Kaiser and Guggenberger, 2000). Examining the nitrogen fractions of the A and AB horizons of a Ferralsol showed that amide structures can also survive into the deeper horizon (Dick et al., 2004) (Fig. 16).

10. Stabilization of organic N by fire

The frequent occurrence of vegetation fires and the debatable use of fire-based management practices, such as the burning of brushwood and litter in woodlands, and of crop wastes on agricultural soils is expected to alter processes involved in stabilization of organic matter and to add substantially to the nitrogen sink. It is clear that fire can destroy the vegetation and most of the soil- or peat-inhabiting organisms. While bog fires can result in a considerable decrease of peat (Rollins et al., 1993), the total content of organic matter in mineral soils can also increase due to the accumulation of charred plant necromass after vegetation fires (Almendros et al., 1990). Previous studies on natural ecosystems, as well as laboratory experiments simulating the effect of fire

on natural or synthetic soils, showed that pyromorphic humus consists of rearranged macromolecular substances that are highly aromatic in nature (Almendros et al., 2003) and consist of mostly heteroaromatic-N (Knicker et al., 1996c). Solid-state ^{13}C and ^{15}N NMR spectroscopic studies on a peat that was heated at 350°C for up to 120 s demonstrated that the increased concentrations of aromatic C-types are not only caused by a relative enrichment concomitant to the selective thermal degradation of labile structures (mainly *O*-alkyl) but correspond to newly synthesised structures that were formed from aliphatic materials (Almendros et al., 2003). It was further shown that the heterocyclic N-forms in the charred residues derived from heat induced transformation of peptides rather than from the selective enrichment of more heat stable pyrrolic forms present in the untreated peat.

This charred organic material is expected to increase resistance to biological degradation. Thus, charred plant material can have a considerable contribution to refractory organic matter in soils that have been exposed to fires and to sediments through input via rivers and precipitations. This assumption was supported by Skjemstad et al. (1996). Subjecting the residues after UV-photooxidation of some Australian soils to scanning electron microscopy and solid-state ^{13}C NMR spectroscopy, up to 30% of the soil carbon of some of these samples was identified as char (Skjemstad et al., 1996). Analyzing the residues by solid-state ^{15}N NMR spectroscopy confirmed the presence of pyrrolic-N, although considerable intensity remained in the chemical shift region of amides (Knicker and Skjemstad, 2000). Applying hydrolysis with 6 M HCl revealed an amino acid N content of 12% to 15% of the UV-photooxidation resistant organic N. Those amino acids may have survived UV-photooxidation by incorporation into microaggregates that remained unaffected by the treatment. On the other hand, entrapment of peptides in char particles occurring during incomplete thermal degradation of the plant residues during char production may represent an alternative explanation for their protection.

The accumulation of heterocyclic N was also observed in the topsoil of a Dystric Xerochrepts from the Sierra de Aznalcóllar (Southern Spain) which was affected by fire, 5 years before sampling (Fig. 17). Amide N is still the dominant form. This may indicate

Fig. 17. Solid-state ^{15}N NMR spectra of a fire affected and a fire unaffected topsoil of Dystric Cambisols from the Sierra de Aznalcóllar, Spain. The fire-affected soil took 5 years after the fire event.

that not all peptide structures of the necromass are transformed by the fire, but it is also possible that those amides became part of melanoidized compounds, known to be formed during thermal treatment of sugar and amino acid containing mixtures. The fact that pyrrole-type N remained undetected in the solid-state ^{15}N NMR spectra of fire-unaffected soil organic matter supports its pyrogenic origin. Thus, pyrrole identification in soil organic matter by means of solid-state ^{15}N NMR spectroscopy may be taken as a molecular marker for the presence of pyromorphic humic material in the soil.

11. Conclusions

Summarizing the presented results, a major finding is that amides, most likely deriving from proteinaceous material represents the major fraction of the organic nitrogen in soils and recent sediments. The fact that the samples were obtained from locations, differing in the chemical composition of the input

material and exhibiting different physical and chemical conditions during formation, strongly points to the assumption that the survival of amides is a ubiquitous phenomenon and exists even into the stage of fossilization of organic-matter-rich deposits. Encapsulation into refractory biopolymers or entrapment within stabilized organic residues seems to be the major stabilization mechanism of organic N in mineral depleted deposits. In soils, but also in sediments rich in mineral matter, however, association of originally labile organic N with minerals may be an additional protection pathway. Transportation and adsorption behavior of organic N during leaching will further determine their fate. With the results, collected up to now, for mineral matter rich environments none of those stabilization mechanisms can be discriminated or favored and it may be speculated that they can occur simultaneously. Their influence on the protection of organic nitrogen, on the other hand, may vary depending on the physical and chemical condition. To reveal their real impact certainly should be a major focus of future research, if the understanding of organic nitrogen sequestration in soils and sediments is attempted.

A further important finding of the presented research is that the formation of heteroaromatic N has no major impact on organic nitrogen stabilization in any of the so far studied soils and sediments in which biotic degradation dominates over abiotic transformation of biogenic precursors. However, based on the study of the humification of TNT, condensation of N-groups on soil organic matter via amide bonds cannot be excluded. Only in environments, where temperature and pressure have replaced the influence of microbial activity, i.e. during catagenesis or burning, heteroaromatic-N was detected. Such compounds are possibly formed during diagenetically or temperature induced cyclization and rearrangement. In sediments, the timing of the shift in nitrogen functionality from primarily amides to pyrrole analogs is an important question for understanding oil and coal formation processes. First results indicated that this may occur just before fossilization. Further studies, i.e. artificial coalification and additional systematic analysis of sediments with increasing maturation, have to bring more light on the mechanisms and the timing involved in organic nitrogen fossilization.

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