4 Humification and Mineralization in Soils

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1
Definitions and Introduction

Organic matter is the second most important constituent in soils next to the mineral phase. Traditionally, organic matter is subdivided into nonhumic substances and humic substances. The former encompass all nonaltered or weakly altered plant materials that are still morphologically identifiable and are composed of defined biomolecules. In contrast, humic substances represent strongly altered organic materials which do not show macroscopically identifiable structures. The process that leads to formation of humic substances is called humification. As can be seen in Table 1, the definition of this process is quite vague compared to other processes related to organic

Table 1. Definitions of key processes associated with organic matter cycling in soils

Term	Definition
Litter deposition	Input of plant residues onto (surface litter) or into (root litter) soils
Transformation	Transfer of organic carbon within a distinct chemical structure to another chemical structure caused by enzymatic attack or chemical reactions
Decomposition	Breakdown of organic macromolecules into smaller organic molecules and inorganic constituents of organic matter; this process is usually mediated by micro-organisms and includes depolymerization and oxidation reactions
Mineralization	Microbial conversion into inorganic constituents of organic matter
Humification	The process whereby the carbon of organic residues is transformed and converted to humic substances through biochemical and abiotic processes
Partitioning	Splitting of organic matter into different pathways of decomposition
Assimilation	Incorporation of organic carbon in microbial biomass; the product is resynthesized organic matter
Stabilization	Decrease in the potential of organic matter to be mineralized due to biological, chemical, or physical processes

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matter cycling in soil. This is due to the fact that, in contrast to, e.g., mineralization and decomposition, there is often no well-defined product of the metabolic activities of soil microorganisms or the catalytic activities of soil minerals associated with the formation of humic substances (Zech and Kögel-Knabner 1994). On the one hand, this can be assigned to the heterogeneity of organic substances formed by humification processes in terms of their source, chemical and physical composition, and variability within time and space. On the other hand, humic substances often resist analytical identification of their individual compounds. Nevertheless, the advent of modern analytical techniques such as chemolytic techniques coupled with gas chromatography-mass spectrometry (GC-MS), analytical pyrolysis, and nuclear magnetic resonance spectroscopy (NMR) provided important insight into the humification processes (Hedges et al. 2000). Since the last decade, substance-specific analysis of the ^{13}C content of a sample (δ^{13}C) using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) and pyrolysis-IRMS allows a more detailed investigation of the carbon flow through soils and a coupling of processes to rates at which they are taking place (Gleixner et al. 2001).

The objective of this chapter is first to summarize current knowledge on the origin and composition of the organic residues entering soils. Based on this information, decomposition processes associated with the different types of organic materials and the humification processes related to these are discussed. Because a wealth of information about decomposition and humification processes can be found in numerable text books, here primarily current concepts are discussed. Following the tradition in soil organic matter research, the terms humification and humic substances are used throughout this chapter. However, as will be elucidated in this text, there may be some doubt upon the usefulness of these terms.

2
Soil Organic Matter Resources

In most soils, plant litter materials provide the primary resources for organic matter formation in soil. Exceptions are soils of extreme ecosystems such as dry and cold deserts, where lichens composed of photoautotroph cyanobacteria (green algae) and fungi can form soils crusts (see Chap. 15) and thus represent primary carbon resources for those soils. Also, microorganisms in soils capable of nonphotoautotroph carbon fixation must be considered as primary resources. However, their contribution to total carbon input into soils is probably minor. In addition to the amount of plant litter, its composition and its properties are essential controlling factors for organic matter formation and humification processes in soils

(Kögel-Knabner 2002). Predictors for plant litter decomposition include its content in nitrogen, cellulose, hemicellulose, lignin, and tannins. During decomposition processes, primary resources are partly mineralized and partly assimilated by microorganisms. After death of the microorganisms, the resynthesized organic substances enter the soil organic matter pool as secondary resources. According to Haider (1992), this represents an important parent material for humus formation. Feces and residues of the soil fauna belong to the secondary resources as well. The soil fauna plays an essential role in controlling litter decomposition in soil (Wolters 2000). However, as the relative input of faunal carbon to the soil organic matter pool is low, the part of parent material for humification attributable to animals is much smaller than that of plant and microbial residues.

2.1
Plant Compounds

Plant tissues include intracellular storage materials and structural components that occur in membranes, extracellular or as cell wall constituents (Kögel-Knabner 2002). The storage materials of plants are more easily degradable than the structural components and thus are important carbon and energy sources for microorganisms. Therefore, decomposition of plant materials usually starts within the cell, leaving cell wall materials intact for a longer time (Olah et al. 1978). This variable accessibility to microbial attack can be explained by the different chemical and physical composition of storage and structural materials.

Intracellular and storage materials are primarily composed of proteins, starch, fructans, chlorophyll and other pigments. Proteins represent the most abundant substance group in plant cells and are usually composed of 20 basic, neutral, or acidic amino acids. They can be decomposed by a multitude of microorganisms and are considered to be very bioavailable (Martin and Haider 1986). Starch is an important storage polysaccharide in vascular plants, and consists of two different polymers of glucose, amylose and amylopectin. As polysaccharides are part of the energy metabolism, starch can be rapidly decomposed by aerobic and anaerobic microorganisms. Another intracellular component is chlorophyll which is present in all photosynthetically active cells. Decomposition of chlorophyll already starts during leaf senescence. Products such as the carotenoids and anthocyanins are degradable, however, their fate and relevance in soils are largely unknown (Kögel-Knabner 2002).

The major structural component of cell walls is cellulose. Although 85% of the cellulose has a fibrillar structure with crystalline properties, it is easily degradable by many microorganisms including fungi and eubacteria,

in particular under aerobic conditions. Cellulose fibers are surrounded by noncellulosic polysaccharides, often summarized as hemicelluloses. They are formed by pentose, hexose, hexuronic acids and desoxyhexose monomers, are branched and have a lower degree of polymerization than cellulose. Hemicelluloses are often encrusted with lignin, which is more resistant to metabolic breakdown. This encrustation protects also the hemicellulose from rapid decomposition (Paul and Clark 1996). Lignin stabilizes plant tissues during growth and is specific to vascular plants. It consists of three different alcohols from the C_6–C_3 pool, coumaryl, coniferyl, and sinapyl alcohol. These monomers are embedded in a three-dimensional network by polymerization of free radicals, which contains a multitude of ether and C–C bondings. The involvement of free radicals in the formation of lignin and the resulting disordered molecular structure of lignin is an important reason why only highly specialized organisms (i.e., white rot fungi) are capable of completely breaking down this biomolecule. Lignin decomposers have lignin peroxidase, manganese peroxidase, and laccase that catalyze biological oxidation of lignin by free oxygen radicals. Therefore, lignin is not degraded under anaerobic conditions and accumulate in peats (Kirk and Farrel 1987). Under aerobic conditions, lignin is mineralized completely, but the carbon is not used by microorganisms for metabolic reactions (Haider 1992). Like lignin tannins are also produced via the C_6–C_3 pool. They are polyhydroxyaromatic acids, especially gallic or ellagic acid, are part of the chemical defense system of plants and are considered to be resistant to microbial decomposition. However, little is known in detail about the metabolism and turnover rate of tannins in plant residues (Harborne 1997).

Lipids are a heterogeneous group of substances in plant and microbial tissues and comprise long-chain molecules such as fatty acids or alcohols and branched terpenes including glycerides and related compounds. Waxes, in particular cutin and suberin, are polymerized and cross-linked structures of hydroxy fatty acids containing an even number of carbons in the range from C_{16} to C_{26}. Cutin is found on the outer surface of plant tissue (cuticle) while suberin is a cell wall component of cork cells. Some plants contain also cutan and suberans, nonhydrolyzable biomacromolecules which consist of polymethylene chains in addition to the hydrolyzable polyester material of cutin and suberin, leading to a highly cross-linked structure (Tegelaar et al. 1989).

2.2
Microbial Compounds

Microorganisms mineralize not only all primary resources to inorganic end products, but utilize most plant-derived materials to resynthesize their own biomass. Microbial cell wall materials are a more important source of soil organic matter than the cytoplasm, since organic constituents of the latter are rapidly degraded. While fungi use chitin, glucan, or cellulose to form their cell walls, bacteria use more complex materials, such as glycolipids, peptidoglycans (Fig. 1), proteoglycans, and glycoproteins (Gleixner et al. 2001). Glycolipids are composed of carbohydrates and lipids, whereas pep-
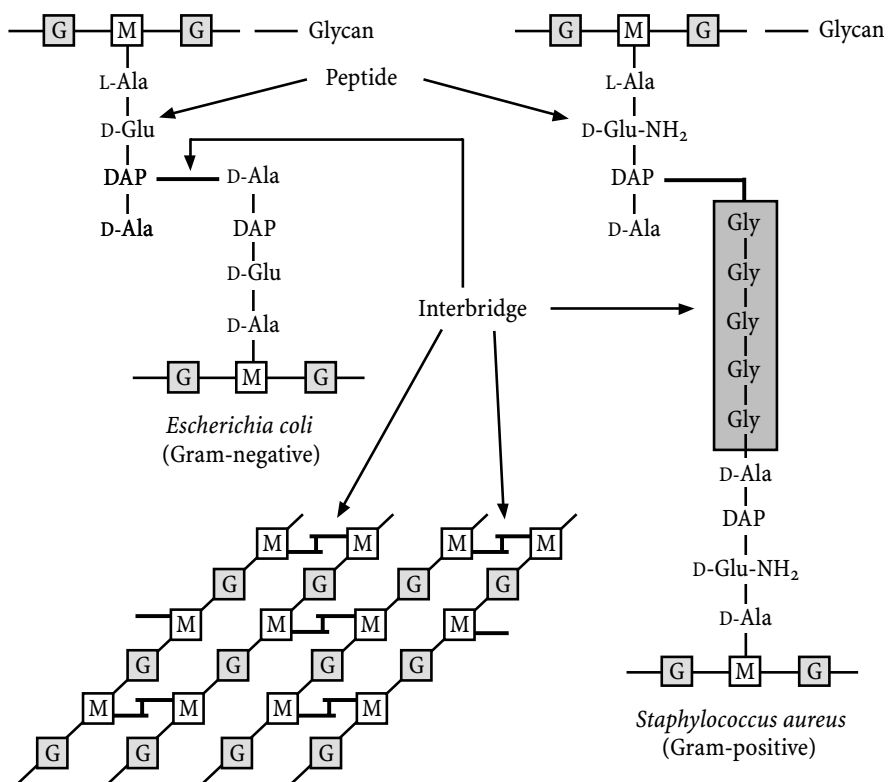

Fig. 1. Schematic representation of the structure of bacterial peptidoglycan (after Madigan et al. 1997). The two images illustrate the linkages of structural units within peptidoglycans of species of Gram-negative and Gram-positive bacteria. The network to the *lower left* shows how these units are assembled into a peptidoglycan sheet (peptide cross-links in *bold*) that is relatively resistant to biodegradation. *G* N-acetylglucosamine, *M* N-acetylmuramic acid, *DAP* meso-diaminopimelic acid, *Ala* alanine, *Gly* glycine, *Glu* glutamic acid. (Hedges et al. 2000)

tidoglycans, proteoglycans, and glycoprotein consist of amino acid polymers and nitrogen-containing sugars (amino sugars) or chitin. The latter three are high molecular weight compounds with a rigid, gel-like structure. Little is known about their fate in soils. However, large concentrations of easily degradable amino sugars released upon acid hydrolysis of soil suggests that these amino sugars are probably well stabilized within the highly cross-linked peptidoglycan after death of the microorganisms (Guggenberger et al. 1999). An additional compound in many fungi, and also some bacteria, are melanin pigments. Melanins consist of a polymeric core of phenolic, indolic, quinone, hydro-quinone and semi-quinone monomers and are non-hydrolyzable (Butler and Day 1998). They are black to brown-colored and are assumed to represent precursors of humic substances in soil (e. g., Saiz-Jimenez 1996). The detailed structure of melanins and rates and pathways of their decomposition are poorly understood. Outside the microbial cell wall extracellular polmeric substances similar to the cell wall components often produce a "diffusion space" that anchors exoenzymes (Gleixner et al. 2001). However, the contribution of these compounds to the pool of microbially derived organic matter in soil is not known.

2.3
Black Carbon

Another type of carbon source in soils is the organic remains of fires, such as charcoal and soot. Due to the black color of such substances, they are referred to as black carbon. Charcoal is the highly condensed, aromatic solid residue of the biomass burned and often still holds the morphological properties of the burned biomass. In contrast, soot is generated de novo in the gas phase of a fire (Kuhlbusch and Crutzen 1995). Soot consists of multilayers of highly condensed aromatic structures that are randomly oriented or well ordered, forming three-dimensional "onion-type" structures (Fig. 2). Fires occur in almost all ecosystems due to lightning or anthropogenic activities, and soot is distributed in the atmosphere worldwide. Hence, black carbon is a ubiquitous carbon species in soils (Goldberg 1985). Estimated global black carbon production is 0.04–0.6 Gt from vegetation fires and 0.007–0.024 Gt from fossil fuel combustion (Kuhlbusch and Crutzen 1995). Quantification of black carbon is difficult because its chemical structure and the physical size of it are extremely heterogeneous. However, in fire ecosystems, black carbon can account for up to 60% of total soil organic carbon (Skjemstad et al. 1996). In soils close to coal processing industries, the contribution of black carbon can even be up to 80%. Hence, black carbon must be considered as an important source of soil organic matter in many ecosystems.
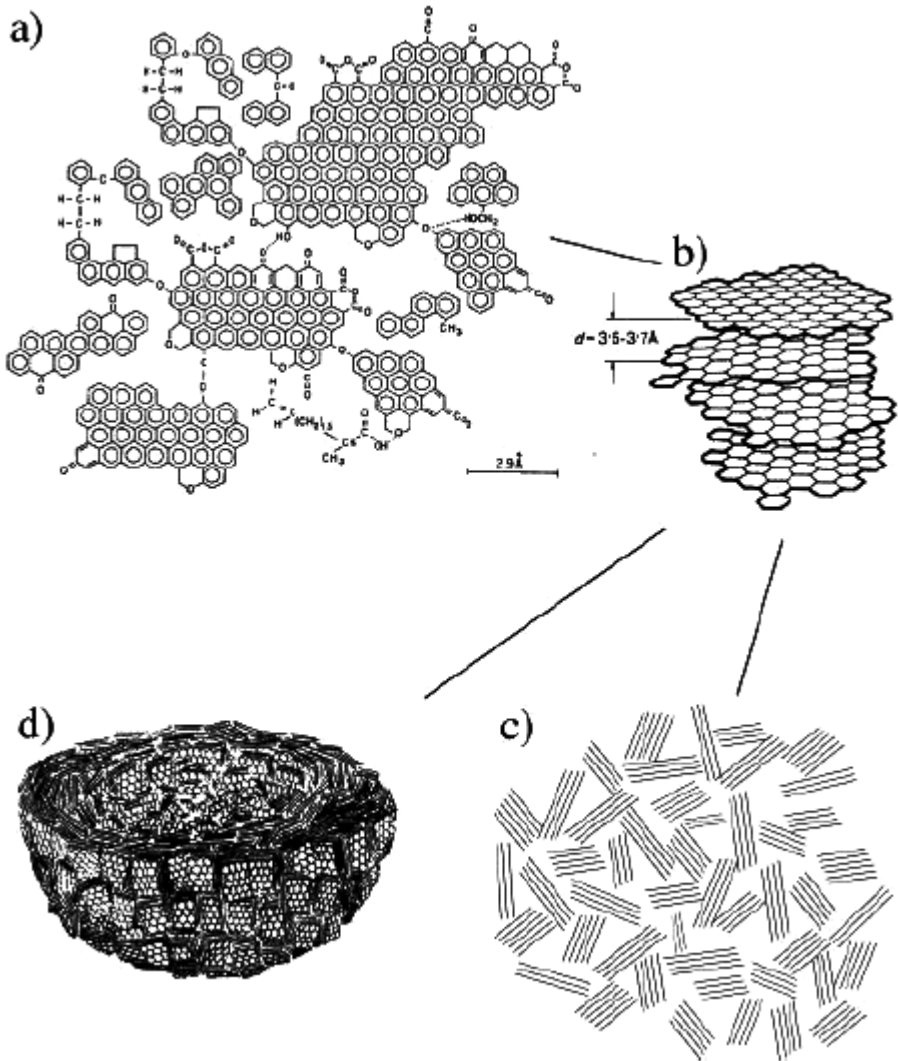
Fig. 2. Soot structure as **a** produced in the laboratory, **b** forming basic structural units of three to four layers, **c** randomly oriented basic structural units shown as a two-dimensional schematic diagram, **d** onion-type particle with several condensation seeds. (Gleixner et al. 2001)

3
Mineralization and Humification Pathways

Fresh litter is very different from older, decomposed litter and organic matter associated with the mineral soil from a chemical point of view. These chemical modifications are a result of primarily biotic decompo-

sition and humification processes occurring in the soil. Vice versa, the different chemical composition influences the rate-regulating factors of organic matter decomposition and the size, composition, and activity of the microbial community.

3.1
Factors Affecting Decomposition and Mineralization

Decomposition of organic residues is the progressive dismantling of organic materials, ultimately into inorganic components, which will then be defined as mineralization (Gregorich and Janzen 2000). Decomposition is mediated mainly by soil microorganisms which derive energy and nutrients from the process. The net effect is the release of carbon and nutrients back into biological circulation.

Decomposition of organic matter is strongly influenced by environmental factors such as water potential, oxygen supply, temperature, nutrient supply and pH, and the quality of organic resource. Mostly, these factors are coupled and cannot be separated from the acting decomposer organisms, the soil fauna and finally the microorganisms (Fig. 3). The three groups of factors: environment, resource quality, and decomposer organism, not only determine the rate of the process, but also the final products of decomposition. The substrates for decomposition and mineralization include a wide range of materials, forming a continuum from fresh primary and
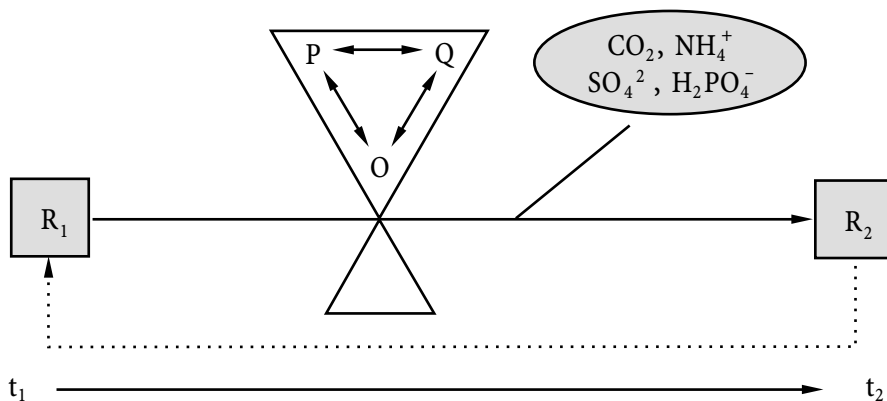

Fig. 3. The decomposition process is regulated by three groups of factors: the physical-chemical environment (P), the quality of the resource (Q), and the acting decomposer organisms (O). The organic resource is changed in this from R_1 to R_2 over time, t_1 to t_2. The changed resource R_2 enters the decomposition cascade where it is further decomposed to $R_3\ldots R_x$ and redistributed through comminution, catabolism and leaching. (Modified from Gregorich and Janzen 2001)

secondary residues to very stable, humified organic matter. For simplicity, decomposition can be subdivided into primary decomposition, involving the breakdown of fresh litter, and secondary decomposition, involving the progressive breakdown of humified organic matter (Gregorich and Janzen 2000).

Of the environmental factors the water potential affects decomposition because soil microorganisms depend on water for survival and mobility. Optimum soil water potentials for decomposition of organic residues are between -0.01 and -0.05 MPa, however, fungi tend to tolerate lower water potentials of -4 to -10 MPa (Sommers et al. 1981). At water potentials higher than about -0.01 MPa, decomposition is slowed by a low oxygen supply. This situation is typical in peatlands where under anaerobic conditions the metabolism of microorganisms shifts to less energy-efficient fermentation or to nitrate and sulfate reduction and methane production. Under these conditions, breakdown of aromatic and phenolic substances such as lignin, which requires oxygen, is not possible and these compounds accumulate (Haider 1992). The second important abiotic driving variable is the temperature. High temperatures in tropical regions lead to a rapid decomposition of organic matter, whereas in boreal regions cold winters and hot and dry summers restrict biomass production and decay. In temperate regions with a well balanced water supply, the decomposition rate of organic residues is high, unless it is limited by the nutrient supply of the soil or litter quality, i. e., conifer litter.

The substrate quality, i. e., the composition of the different primary and secondary resources as described above, is an intrinsic property that affects decomposition. Destabilization of molecules occurs when the activation energy needed for bond breaking is available. As a rough estimate, double and triple bonds are most stable, followed by homopolar C–C and C–H bonds, whereas heteropolar C–O and C–N bonds are least stable (Gleixner et al. 2001). In biological systems, the activation energy is lowered by specific enzymes that catalyze the breakdown of molecules, and often whole sets of enzymes are involved in decomposition of biomolecules in soils. A critical factor in enzymatic degradation is that most microorganisms are unable to transport molecules larger than about 600 Da through their cell walls. Hence, they must be cleaved to smaller molecules outside the cell by exoenzymes. Further, the enzymes need a specific molecular environment. The substrate must fit exactly into the active center of the enzymes. In polymeric biomolecules, access of the enzymes to their specific reaction sites of the substrate is often impeded. These interactions are illustrated in a classical experiment of Haider and Martin (1975) where mineralization of ^{14}C-labeled monomeric and polymeric substances are studied (Fig. 4). Carbohydrates and proteins are degraded rapidly, being partly mineralized and partly used for microbial biomass formation. Monomeric lignin con-
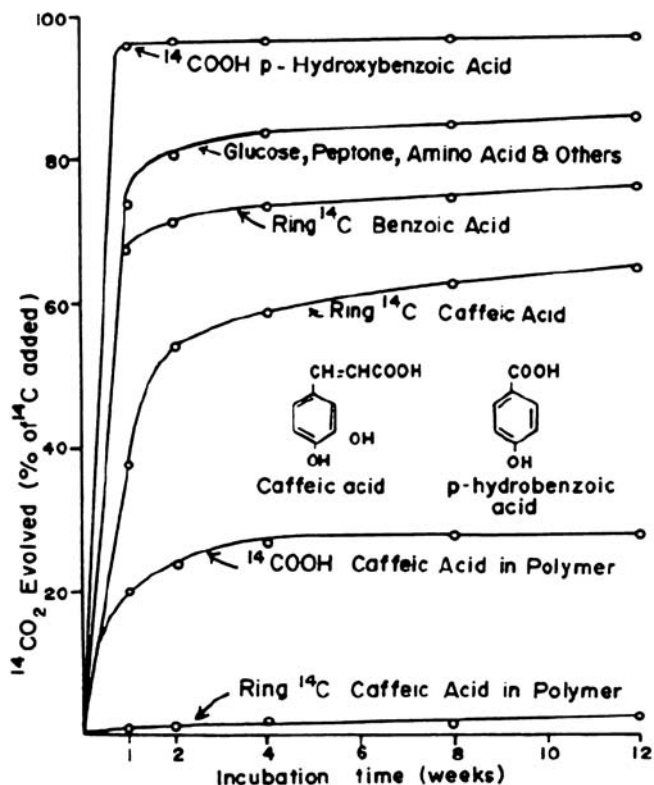
Fig. 4. Decomposition of ^{14}C-labeled lignin moieties. (Haider and Martin 1975)

stituents, even if located in the aromatic ring structure, are also mineralized quite rapidly. In contrast, the stability of lignin constituents is much higher when located in the polymer. The polymeric and disordered structure of lignin prevents rapid enzymatic degradation of this biomolecule. Further, depolymerization of lignin is a co-metabolic process and labile organic carbon species are needed as a co-metabolic substrate, because there is no energy gain during lignin decomposition (Haider 1992). However, recent studies using ^{13}C NMR spectroscopy and analytical pyrolysis have shown that lignin is altered relatively rapidly in soils and does not appear to be stabilized in the long term (Kögel-Knabner 1993; Gleixner et al. 2002). In forest soils, intact lignin structures decrease considerably with increasing soil depth (Kögel-Knabner 1993), which matches the highest potential and diversity of laccase genes in the upper horizons quite well (Luis et al. 2004).

Aliphatic biomacromolecules such as suberans and cutans are also resistant to oxidative and enzymatic attack in soil (Tegelaar et al. 1989), due to reduced mobility of the molecules and blocking of reactive sites (e.g.,

beta position). For black carbon, the resistance against biodegradation is due to its strongly condensed and disordered molecular structure and the high degree of internal cross-linkages (Almendros and Dorado 1999). Only enzymatically catalyzed reactions forming small radicals from highly polymeric lignins, biomacromolecular aliphatics, and black carbon are able to degrade these substances. These products are further mineralized, but they are also believed to serve as basic units for abiotic spontaneous condensation reactions. The latter process is considered to represent one pathway in the formation of humic substances (Hedges 1988).

3.2
Humification Processes

Soil humic substances and the associated processes of humification have been a subject of intense research in soil science for more than two centuries (Archard 1786). Despite these tremendous efforts, formation of humic substances is probably still the most vague field in soil science and subject to many myths. This problem is mainly due to the long-time inaccessibility of humic substances by analytical methods. Still nowadays, the structural elucidation of humic substances is a challenging task. Nevertheless, the identification of changes in the chemical and physical form of the organic compounds from primary and secondary resources to humic substances is an important prerequisite in the identification of humification pathways. In the following, this will be critically elucidated.

3.2.1
Definition and Composition of Humic Substances

Classically, soil humus is composed of humic substances and nonhumic biomolecules of plant and microbial origin after dissolved organic carbon (DOC) and macroorganic matter has been removed by water extraction and particle size and density fractionation (Oades 1989). Humic substances and nonhumic biomolecules cannot be separated by chemical extraction, and both types of organic materials can be fractionated into fulvic acid, humic acid and the residual humin, based on their solubility in aqueous alkaline and acid solutions. There is criticism that the separation of organic matter into these humic fractions may involve some source of bias (Baldock and Nelson 2000), including (1) the questionable ability of the alkaline extractable organic matter to represent the composition of the nonextracted organic fractions, (2) differences in the chemical characteristics in extracted organic molecules with those of the same materials existing in soils in an adsorbed state, and (3) the formation of artifacts during

the extraction procedure. The largest drawback of this concept is that the solubility-based organic matter fractions cannot be related to microbial turnover rates in contrast to fractions that are physically isolated based on size or density characteristics (Christensen 1996). Nevertheless, particular modern spectroscopic techniques to study the chemical structure of humic substances, such as ^{13}C NMR spectroscopy and analytical pyrolysis, provided important insight into the composition of humic substances. It is important, however, that the analytical limitations of these, as in any other degradative and nondegradative technique to study the structure of organic substances, are considered in a proper interpretation of the results (summarized in Guggenberger 2002).

In recent decades, a respectable number of review articles (e. g., Oades 1989; Kögel-Knabner 1993; Preston 1996) and textbooks (Aiken et al. 1985; Hayes et al. 1989; Stevenson 1994) used information from these different analytical techniques to present detailed information on the chemical and physical structure of humic substances. Baldock and Nelson (2000) summarized the results of the chemical composition of humic substances as follows: (1) aromatic rings are a significant component and multiple substitutions with carboxyl, hydroxyl, carbonyl and alkyl groups exist; (2) significant quantities of C1–C20 alkyl C chains either unsubstituted or substituted with O-containing functional groups are present; (3) aromatic and alkyl groups are bound together principally at random by C–C bonds and ether linkages to form the backbone structure of humic substances; (4) simple and polymeric proteinaceous and carbohydrate groups are associated with this backbone. These numerous substances being different in chemical composition, size, and type of bonding suggest that a variety of different mechanisms may be involved in their genesis, which will be discussed next.

3.2.2
Formation Pathways of Humic Substances

One of the proposed pathways of humic substance formation is the spontaneous "heteropolycondensation" reaction between small reactive intermediates released during enzymatic breakdown of biomacromolecules (Hedges 1988). These processes are extracellular and may be catalyzed by microbial exoenzymes. Such humification theories are based on the observation that many simple organic molecules (e. g., amino acids, phenols and sugars) abiotically condense to produce extremely complex assemblages of molecules that exhibit the brown color and many of the physicochemical properties of soil organic matter (Hedges et al. 2000). These condensations include Maillard (or "browning") reactions between carbohydrates and amino acids that form dark, often aromatic, melanoidins (Benzing-Purdie

et al. 1983). In soils, such compounds have been identified primarily within foodstuffs of archaeological sites (Evershed et al. 1997). In the polyphenol theory, monomeric phenolic species are produced by enzymatic degradation of lignin. They are capable of forming a quinone structure in the presence of oxygen or polyphenoloxidase enzymes, which spontaneously polymerize with each other or with amines or ammonia to produce polymeric compounds (Baldock and Nelson 2000). Phenols and quinones can also be formed from carbohydrates (Hedges 1978). The heteropolycondensation theory includes also photo-oxidation of polyunsaturated lipids. One argument that is often raised in favor of the heteropolycondensation pathway is the strong resemblance of artificial humic substances produced from well-defined precursors in the laboratory with soil humic substances. However, for the heteropolycondensation pathway, usually an environment of high temperature, high pH, and/or UV radiation is needed that is rarely found in soil.

An important argument against abiotic or enzymatically catalyzed heteropolycondensation comes from ^{15}N NMR spectroscopy. In soils and modern sediments, organic nitrogen is primarily in the amide form (Knicker et al. 1996; Knicker and Hatcher 2001), whereas in ancient sediments aromatic heterocycles are typically found (Derenne et al. 1998), which are probably the product of advanced abiotic condensation (melanoidins). In the ancient sediments, this abiotic formation of aromatic nitrogen can be explained by diagenetic processes. However, in soils, the formation of amides is difficult to explain by abiotic processes. The predominance of amides points strongly toward a remnant biochemical component of the humic substances. Hedges et al. (2000) reported another argument against abiotic heteropolycondensation: extensive in situ formation of new chemical compounds is seldom evident from either NMR (Fig. 5) or molecular-level analyses. It can thus be concluded that during organic matter degradation, relatively resistant biochemicals are selectively concentrated into humic substances implying the concept of selective preservation.

For many aerobic soils, application of NMR and molecular-level analysis showed that the humic substances contain relatively little aromatic carbon and the aromatic carbon present shows little resemblance to lignin (Kögel-Knabner 2000). Hence, it appears that lignin is decomposed rather rapidly under aerobic conditions and is not selectively enriched in humic substances. In contrast, in most soils, the humic materials are rich in highly aliphatic compounds. Possible sources of such aliphatic compounds can be hydrolysis-resistant biomacromolecules in vascular plants, e.g., cutans and suberans (Largeau et al. 1986; Tegellar et al. 1989). Similarly, highly resistant biomolecules have been also identified in microbial and algal cell walls (Derenne et al. 1991; DeLong et al. 1998). Such components may be stabilized against decomposition by spontaneous oxidative cross-linking.
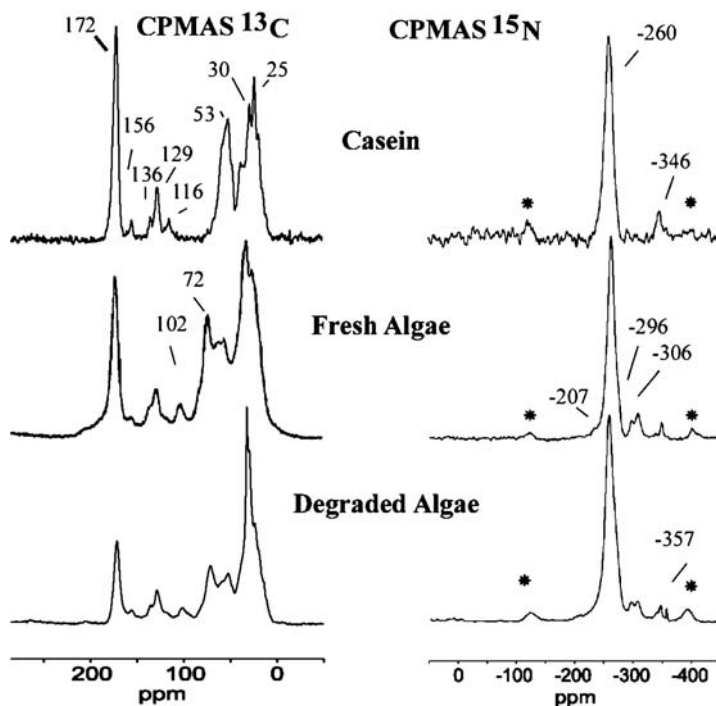
Fig. 5. Solid state CP-MAS ^{13}C and ^{15}N NMR spectra of casein (a milk protein), fresh algae and biodegraded algae. Within the ^{13}C spectra, the chemical shift regions and corresponding major carbon types are: 0–45 ppm (alkyl), 45–60 ppm (*N*-alkyl and methoxyl), 60–95 (*O*-alkyl), 95–115 (di-*O*-alkyl), 115–145 (aromatic), 145–160 (*O*-aromatic) and 160–210 (carboxyl/carbonyl). In the ^{13}C spectra, the predominance of protein in fresh algae is evident, as is the loss of nonalkyl carbon during algal degradation. For the ^{15}N spectra, amide nitrogen (−260 ppm) constitutes the main resonances of all samples. *Asterisks* indicate spinning side bands of larger central resonance. (Knicker 2000)

Such cross-linking and repackaging, however, do not necessarily produce large changes in the bulk chemical composition of the precursor molecule. Even if such resistant biomacromolecules occur only in trace amounts in living organisms, they can be concentrated by degradation to become major components of humic substances in soil.

Besides aliphatic moieties, humic substances are often rich in polysaccharides and nitrogenous organic compounds (Kögel-Knabner 2000; Baldock and Nelson 2000). Hence, humic compounds usually have a C:N ratio of 10 which is much narrower than that of the litter material. As mentioned above, soil organic nitrogen is almost exclusively in the amide form. While it is obvious why resistant aliphatic biomacromolecules are major constituents in humic soil materials, this is not clear for the potentially easily
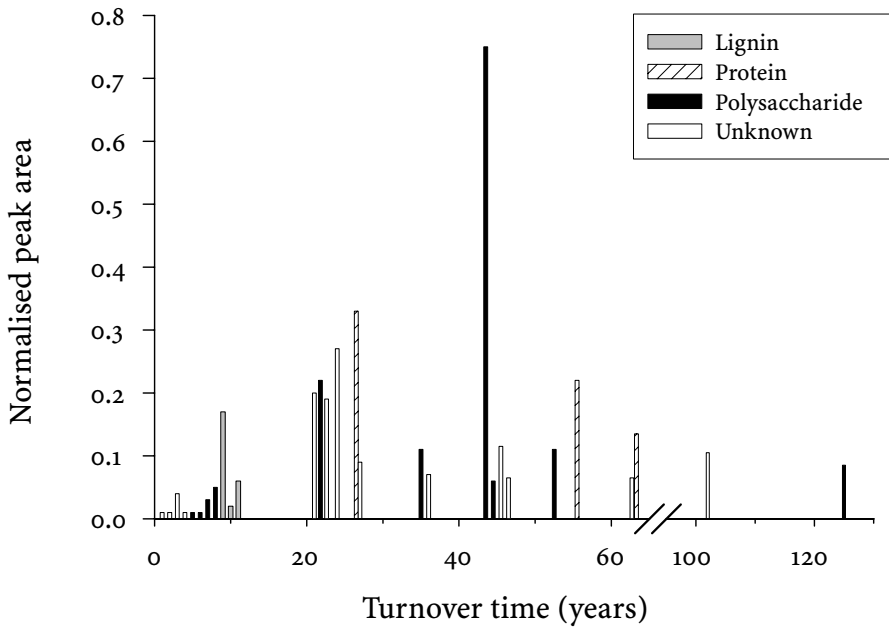
Fig. 6. Peak areas and mean residence time of pyrolysis products from maize leaves, wheat and maize soil. Pyrolysis products are related to their probable precursors. Protein fraction contains pyrolysis products from proteins, amino acid moieties and chitin. Pyrolysis products, only present in maize leaves, are supposed to be degraded within a few years, i. e., all peaks with a turnover under 20 years. In contrast to proteins and polysaccharides, lignin appears to be rapidly degraded in soil. (Gleixner et al. 2002)

degradable polysaccharides and peptides and proteins. However, recently the determination of molecular turnover rates using pyrolysis-isotope ratio mass spectrometry of C3/C4 sequence soils indicated some of the longest turnover rates for pyrolysis products derived from proteins and polysaccharides (Gleixner et al. 1999, 2002; Fig. 6).

One key process that leads to the enrichment of proteinaceous compounds in soil humic substances may be the reduced accessibility of the organic substrate by enzymes. Since most enzymes must be compatible with water, processes that remove water from the immediate surroundings of the macromolecules will slow down their degradation. Folding of large molecules and aggregation of small molecules (Piccolo 2002) can both lead to internal hydrophobic microenvironments whose isolation is strongly favored by the polarity and highly ordered "structure" of the surrounding water network (Hedges et al. 2000). Hydrophobicity, along with hydrogen bonding ion pairing, are strong contributing factors to the complex three-dimensional folding patterns of proteins. Hence, it appears that physical

Cell wall:
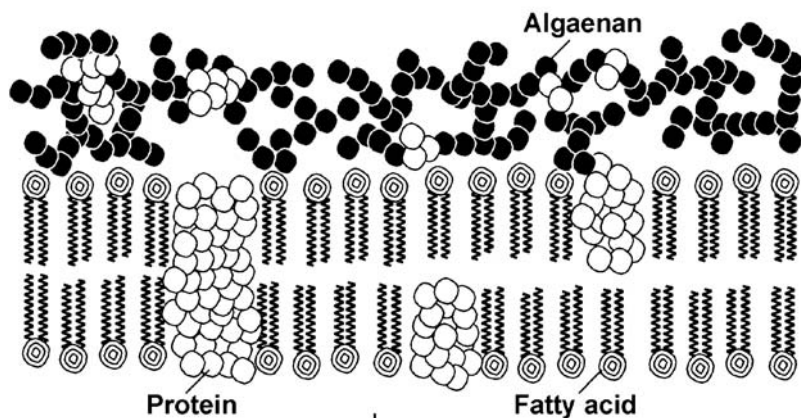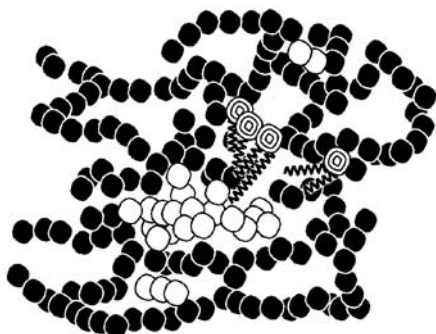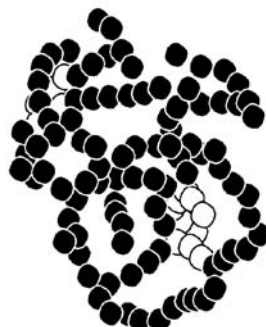
Fig. 7. Schematic illustration of the structure of an algaenan-containing algal cell wall (*above*) and proteinaceous organic matter encapsulated within degraded algal cell wall material (*below*). By encapsulation intrinsically labile proteins and peptides might be physically protected from biodegradation. (Knicker and Hatcher 2001)

availability to attack, rather than intrinsic bonding energies, determines the biological reaction rates and, hence, the processes of selective preservation in humic substances formation. Another example for this is the observation that intrinsically reactive biochemicals such as proteinaceous organic matter appear to be encapsulated in some type of protective matrix (Knicker and Hatcher 1997, 2001; Fig. 7).

In order to react with enzymes, chemical subunits of organic molecules need to be accessible, but they must also be in a very specific alignment.

Relatively subtle chemical or conformational changes leading to minor modifications in low-energy bonding can cause profound changes in enzyme function and stability (Stotzky et al. 1996). Therefore, when portions of proteins are spread over a particle surface they can be resistant to reaction, even though they are physically accessible to enzymes (Nagata and Kirchman 1996). In fact, this process is considered to be primarily responsible for the stabilizing effect of sorption processes on organic substances (Khanna et al. 1998). However, such conformational changes that largely affect the biochemical reactivity of an organic molecule may not be detectable by most bulk and molecular-level analyses.

In addition to the restricted accessibility of enzymes to moderately altered biomolecules, another process may be responsible for large proportions of proteins and polysaccharides in soil humic substances. Microorganisms secrete organic substances, such as enzymes and other biopolymers like polysaccharides and proteins. Baldock et al. (1990) showed by incubating a soil with uniformly labeled ^{13}C-glucose that utilization of glucose by soil microorganisms results predominantly in the synthesis of *O*- and *N*-alkyl C (i. e., polysaccharides and proteins) besides alkyl C. According to molecular isotopic analysis, pyrolysis products derived from microbial polysaccharides and proteins have relatively high mean residence times of several decades (Gleixner et al. 2002). This does not necessarily mean that the distinct molecules are stabilized for this time. Large concentrations of microbial polysaccharides and proteins within the humic substances may be also due to a microbial recycling (Gleixner et al. 2002). Hence, a carbon atom may reside long in soil, but be repeatedly incorporated into polysaccharides or proteins with a more rapid turnover. It could be only the apparent residence time that is long for some polysaccharides and proteins. Such a microbial loop of metabolization, mineralization, and re-metabolization may be considered as an important process in stabilization of soil carbon and reducing the carbon losses to the atmosphere. However, this important process is not covered within the current concepts of humification. Another component that is not included in current humification concepts is black carbon.

3.2.3
The Fate of Black Carbon in Soil

Black carbon derived from pyrogenic processes is found in 65-million-year-old marine sediments (Herring 1985) and was considered for a time to be almost inert in the soil environment (Swift 2001). However, recent carbon and oxygen isotopic studies suggest that black carbon degradation in soils can take less than a century (Bird et al. 1999). Hence, it is not reasonable to assume that black carbon is completely inert, i. e., nonreactive. Photo-

chemical breakdown of black carbon was already identified by Ogren and Charlson (1983). Hamer et al. (2004) showed that artificially charred plant materials could be microbially mineralized (about 0.8% during 60 days) in the presence of an easily available carbon source, thus indicating a co-metabolic pathway. According to Glaser et al. (2000), surfaces of black carbon molecules in soil are highly oxidized and able to react with mineral surfaces. Möller et al. (2000) identified benzenehexacarboxylic (mellitic) acid as a highly altered breakdown product of black carbon in a tropical soil. Since this substance cannot be produced from a biogenic precursor (Glaser et al. 1998), it must be the product of charred plant material. Obviously oxidative degradation of black carbon is taking place, leaving smaller and highly reactive aromatic products behind. Based on the facts that lignin is degraded quite rapidly in aerobic soils and that black carbon can be oxidatively altered, Haumaier and Zech (1995) concluded that biooxidative degradation of black carbon is a possible source of highly aromatic humic acids. Since black carbon is almost ubiquitous in soils, it can be generally asked if this elemental carbon source is the major precursor of highly aromatic substances found in humic fractions. However, this needs to be investigated in the future.

4
Conclusions

It appears that the definitions of humification and humic substances depend on the analytical capabilities to elucidate the structure of these biomolecules. Current concepts of humification largely reject random heteropolycondensation reaction as an important pathway in the formation of humic substances (see also Hedges et al. 2000). With the aid of new analytical instrumentation, a new picture emerges showing that the processes of biodegradation and humification cannot be separated, and that relatively resistant organic molecules are selectively concentrated into humic substances. In particular, aliphatic moieties within the humic fractions show strong resemblance to their mother substances. Often subtle changes in the chemical composition and/or conformational changes are decisive whether a biomolecule is degraded rapidly or preserved against decomposition over a long time. Such conformational changes are likely to occur upon sorption to mineral phases in soil. While such processes of selective preservation received considerable attention in the last two decades, other major points that need to be addressed in the future are:

1. The contribution of rapid resynthesis of microbial biomass (microbial loop) to polysaccharide and proteinaceous moieties in the humic

fractions is unclear. The use of stable isotope techniques may help to understand pathways and efficiency of this process as well as to distinguish between the turnover of a carbon atom in soil from that of a distinct molecule.

2. Black carbon appears to be a major source of humic substances at least in some soils. The degradation reactions are poorly understood, and there is even huge analytical uncertainty in distinguishing unaltered black carbon from its decomposition products showing a different degree of biooxidative alteration.

3. Humic substances isolated from soils of different pedogenesis differ strongly in their chemical structural composition (Kögel-Knabner 2000). Probably, the humification pathways are the same in the different soil environments, but the proportions of the individual pathways may differ. Yet, there is little knowledge about the controlling factors.

Nevertheless, a picture emerges that humification can be increasingly identified as a multitude of specific biochemical and biophysical processes that alter the structural composition of the organic matter. Other important processes that influence the fate of organic matter in soil are not discussed here and include faunal communition, turbation and intestinal degradation, formation of dissolved organic matter, and sorption processes to soil minerals. The interplay of all of these processes lead to different fractions of soil organic matter with different source materials, chemical and physical structural composition, and functions. Many of these fractions are well defined in terms of the process that leads to their formation and in terms of their functions, e.g., dissolved organic matter, free and occluded organic particulate organic matter, mineral-associated organic matter. In contrast, neither humification is an adequate description of specific processes, nor is humic substances a useful soil organic matter pool. It is time to be more specific in the separation, identification, and denomination of distinct processes that are taking place in mineralization, alteration, and stabilization of organic matter in soils. Such vague and poorly defined terms such as humification and humic substances are of little help to improve our understanding of the functioning of organic matter cycling in soils and, therefore, should be abandoned.

References

Achard FC (1786) Chemische Untersuchungen des Torfs. Crell's Chem Ann 2:391–403

Aiken GR, McKnight DM, Wershaw RL, MacCarthy P (1985) Humic substances in soil, sediment and water. Wiley, New York, NY

Almendros G, Dorado J (1999) Molecular characteristics related to the biodegradability of humic acid preparations. Eur J Soil Sci 50:227–236

Baldock JA, Nelson PN (2000) Soil organic matter. In: Sumner MR (ed) Handbook of soil science. CRC Press, Boca Raton, pp B-25–B-84

Baldock JA, Oades JM, Vassallo AM, Wilson MA (1990) Solid state CP/MAS ^{13}C N.M.R. analysis of particle size and density fractions of a soil incubated with uniformly labelled ^{13}C-glucose. Aust J Soil Sci 28:193–212

Benzing-Purdie L, Ripmeester J, Preston C (1983) Elucidation of the nitrogen forms in melanoidins and humic acids by nitrogen-15 cross polarization-magic angle spinning nuclear magnetic resonance spectroscopy. J Agric Food Chem 31:913–915

Bird MI, Moyo C, Veenendaal EM, Lloyd J, Howard DM (1999) Stability of elemental carbon in a savanna soil. Global Biogeochem Cycles 13:923–932

Butler MJ, Day AW (1998) Fungal melanins: a review. Can J Microbiol 44:1115–1136

Christensen BT (1996) Carbon in primary and secondary organo-mineral complexes. In: Carter MR, Stewart BA (eds) Advances in soil science – Structure and organic matter storage in agricultural soils. CRC Lewis, Boca Raton, pp 97–165

DeLong EF, King LL, Massana R, Cittone H, Murray A, Schleper C, Wakeham SG (1998) Cyclic and acyclic dibiphytanyl ether lipids in marine psychrophilic crenarchaeotes: evolutionary and ecological implications. Appl Environ Microbiol 64:1133–1138

Derenne S, Largeau C, Casadevall E, Berkaloff C, Rousseau B (1991) Chemical evidence of kerogen formation in source rocks and oil shales via selective preservation of thin resistant outer walls of microalgae: origin of ultralaminae. Geochim Cosmochim Acta 55:1041–1050

Derenne PS, Knicker H, Largeau C, Hatcher P (1998) Timing and mechanisms of changes in nitrogen functionality during biomass fossilization. In: Stankiewicz BA, van Bergen PF (eds) Nitrogen-containing macromolecules in the bio- and geosphere. ACS Symposium Series 707. American Chemical Society, Washington, DC, pp 245–253

Evershed RP, Bland HA, van Bergen PF, Carter JC (1997) Volatile compounds in archaeological plant remains and the Maillard reaction during decay of organic matter. Science 278:432–433

Glaser B, Haumaier L, Guggenberger G, Zech W (1998) Black carbon in soils: the use of benzenecarboxylic acids as specific markers. Org Geochem 29:811–819

Glaser B, Balashov E, Haumaier L, Guggenberger G, Zech W (2000) Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. Org Geochem 31:669–678

Gleixner G, Bol R, Balesdent J (1999) Molecular insight into soil carbon turnover. Rapid Comm Mass Spectrom 13:1278–1283

Gleixner G, Czimczik C, Kramer C, Lühker BM, Schmidt MWI (2001) Plant compounds and their turnover and stability as soil organic matter. In: Schulze E-D, Heimann M, Harrison S, Holland E, Lloyd J, Prentice IC, Schimel D (eds) Global biogeochemical cycles in the climate system. Academic Press, San Diego, pp 201–216

Gleixner G, Poirier N, Bol R, Balesdent J (2002) Molecular dynamics of organic matter in a cultivated soil. Org Geochem 33:357–366

Goldberg ED (1985) Black carbon in the environment. Wiley, New York

Gregorich G, Janzen HH (2000) Decomposition. In: Sumner ME (ed) Handbook of soil science. CRC Press, Boca Raton, pp C-107–C-119

Guggenberger G (2002) Organic matter structure and characterization. In: Lal R (ed) Encyclopedia of soil science. Marcel Dekker, New York, pp 929–935

Guggenberger G, Frey SD, Six J, Paustian K, Elliott ET (1999) Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Sci Soc Am J 63:1188–1198

Haider K (1992) Problems related to the humification processes in soils of the temperate climate. In: Bollag J-M, Stotzky G (eds) Soil biochemistry, vol 7. Marcel Dekker, New York, pp 55–94

Haider K, Martin JP (1975) Decomposition of specifically carbon-14 labelled benzoic and cinnamic acid derivatives in soil. Soil Sci Soc Am Proc 39:657–662

Hamer U, Marschner B, Brodowski S, Amelung W (2004) Interactive priming of black carbon and glucose mineralisation. Org Geochem 35:823–830

Harborne JB (1997) Role of phenolic secondary metabolites in plants and their degradation in nature. In: Cadisch G, Giller KE (eds) Driven by nature: Plant litter quality and decomposition. CAB International, Wallingford, pp 67–74

Haumaier L, Zech W (1995) Black carbon – possible source of highly aromatic components of soil humic acids. Org Geochem 23:191–196

Hayes MHB, MacCarthy P, Malcolm R, Swift RS (1989) Humic substances II. In search of structure. Wiley-Interscience, Chichester

Hedges JI (1978) The formation and clay mineral reactions of melanoidins. Geochim Cosmochim Acta 42:69–76

Hedges JI (1988) Polymerization of humic substances in natural environments. In: Frimmel FH, Christman, RF (eds) Humic substances and their role in the environment. Wiley, Chichester, pp 45–58

Hedges JI, Eglinton G, Hatcher PG, Kirchman DL, Arnosti C, Derenne S, Evershed RP, Kögel-Knabner I, de Leeuw JW, Littke R, Michaelis W, Rullkötter J (2000) The molecularly-uncharacterized component of nonliving organic matter in natural environment. Org Geochem 31:945–958

Herring JR (1985) Charcoal fluxes into sediments of the North Pacific Ocean: The cenozoic record of burning. Geophys Monogr 32:419–442

Kirk TK, Farrel RL (1987) Enzymatic combustions – the microbial degradation of lignin. Annu Rev Microbiol 41:465–505

Knicker H (2000) Biogenic nitrogen in soils as revealed by solid-state carbon-13 and nitrogen-15 nuclear magnetic resonance spectroscopy. J Environ Qual 29:715–723

Knicker H, Hatcher PG (1997) Survival of protein in an organic-rich sediment – possible protection by encapsulation in organic matter. Naturwissenschaften 84:231–234

Knicker H, Hatcher PG (2001) Sequestration of organic nitrogen in the sapropel from Mangrove Lake, Bermuda. Org Geochem 32:733–744

Knicker H, Scaroni AW, Hatcher PG (1996) ^{13}C and ^{15}N NMR spectroscopic investigation on the formation of fossil algal residues. Org Geochem 24:661–669

Kögel-Knabner I (1993) Biodegradation and humification processes in forst soils. In: Bollag J-M, Stotzky G (eds) Soil biochemistry, vol 8. Marcel Dekker, New York, pp 101–137

Kögel-Knabner I (2000) Analytical approaches for characterizing soil organic matter. Org Geochem 31:609–625

Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34:139–162

Khanna M, Yoder M, Calamai L, Stotzky G (1998) X-ray diffractometry and electron microscopy of DNA from *Bacillus subtilis* bound on clay minerals. Sci Soils 3:1

Kuhlbusch TAJ, Crutzen PJ (1995) Toward a global estimate of black carbon in residues of vegetation fires representing a sink of atmospheric CO_2 and a source of O_2. Global Biogeochem Cycles 9:491–501

Largeau C, Derenne S, Casadevall E, Kadouri A, Sellier N (1986) Pyrolysis of immature torbanite and of the resistant biopolymer (PRB A) from extant algae *Botryoccus braunii*; mechanism of formation and structure of torbanite. Org Geochem 10:1023–1032

Luis P, Walther G, Buscot F (2004) Diversity of laccase gene from basidiomycetes in a forest soil. Soil Biol Biochem 36:1025–1036

Madigan MT, Martinko JM, Parker J (1997) Brock – Biology of microorganisms. 8th edn. Prentice Hall, Upper Saddle River

Martin JP, Haider K (1986) Influence of mineral colloids on turnover rates of soil organic carbon. In: Huang PM, Schnitzer M (eds) Interactions of soil minerals with natural organics and microbes, Vol 17. Soil Science Society of America, Madison, pp 283–304

Möller A, Kaiser K, Amelung W, Niamskul C, Udomsri S, Puthawong M, Haumaier L, Zech W (2000) Forms of organic C and P extracted from tropical soils as assessed by liquid-state ^{13}C- and ^{31}P NMR spectroscopy. Aust J Soil Res 38:1017–1035

Nagata T, Kirchman DL (1996) Bacterial degradation of protein adsorbed to model submicron particles in seawater. Mar Ecol Prog Ser 132:241–248

Oades JM (1989) An introduction to organic matter in mineral soils. In: Dixon JB, Weed SB (eds) Minerals in soil environments, 2nd ed. Soil Science Society of America, Madison, pp 89–159

Ogren JA, Charlson RJ (1983) Elemental carbon in the atmosphere: cycle and lifetime. Tellus 35:241–254

Olah G-M, Reisinger O, Kilbertus G (1978) Biodégradation et humification. Atlas ultrastructural. Presses de l'Úniversité Laval, Quebec

Paul EA, Clark FE (1996) Soil microbiology and biochemistry, 2nd edn. Academic Press, San Diego

Piccolo A (2002) The supramolecular structure of humic substances: a novel understanding of humus chemistry and implications in soil science. Adv Agron 75:57–134

Preston CM (1996) Applications of NMR to soil organic matter analysis: history and prospects. Soil Sci 161:144–166

Saiz-Jimenez C (1996) The chemical structure of humic substances: recent advances. In: Piccolo A (ed) Humic substances in terrestrial ecosystems. Elsevier, Amsterdam, pp 1–44

Skjemstad JO, Clarke P, Taylor JA, Oades JM, McClure SG (1996) The chemistry and nature of protected carbon in soil. Aust J Soil Res 34:251–271

Sommers LE, Gilmour CM, Wildung RE, Beck SM (1981) The effect of water potential on decomposition processes in soils. In: Parr JF, Garnder WR, Elliott LF (ed) Water potential relations in soil microbiology. Soil Science Society of America Special Publication 9, Madison, pp 97–117

Stevenson FJ (1994) Humus chemistry. Genesis, composition, reactions. 2nd edn. Wiley, New York

Stotzky G, Gallori E, Khanna M (1996) Transformation in soil. In: Akkermans ADL, van Elsas JD, de Bruijn FJ (eds.) Molecular microbial ecology manual. Kluwer, Dordrecht, pp 1–28

Swift RS (2001) Sequestration of carbon by soil. Soil Sci 166:858–971

Tegelaar EW, de Leeuw JW, Saiz-Jimenez C (1989) Possible origin of aliphatic moieties in humic substances. Sci Total Environ 81/82:1–17

Wolters V (2000) Invertebrate control of soil organic matter stability. Biol Fertil Soils 31:1–19

Zech W, Kögel-Knabner I (1994) Patterns and regulation of organic matter transformation in soils: litter decomposition and humification. In: Schulze ED (ed) Flux control in biological systems: from the enzyme to the population and ecosystem level. Academic Press, New York, pp 303–334