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PLANT COMPONENTS AND SOIL ORGANIC MATTER*

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INTRODUCTION

Large amounts of cellulose, aromatics, and proteinaceous compounds are introduced into the geochemical carbon pool as products of biological activity, or by economic and public health endeavors. Industrial wastes, pesticides, and detergents belong to the latter category. A net dry matter production of 1 kg/m^2, representing about 4000 k. cal of energy, is attained in a significant portion of temperate terrestrial ecosystems (MacFayden, 1967). Most of the annual net primary productivity falls to the surface of the soil or is present as roots and is decomposed there. It is not surprising, therefore, that nearly every naturally produced organic compound can be isolated from the soil in at least small concentrations.

The ability of multicellular plants and animals to cleave the aromatic ring appears to be restricted, most phenolics merely being modified by these organisms which do not seem to contribute substantially to the cyclization of aromatic carbon. Microorganisms, alone and in association with the soil fauna, bear the burden of releasing this carbon. Soil, being the major geostation in the cycling of terrestrial elements, is the site of greatest activity (Towers, 1964; Odum et al., 1967).

Plant and animal residues with an adequate content of nutrients and a fairly low lignin content are rapidly degraded. The rate of decomposition is dependent on the moisture content, aeration, and temperature of the soil. It is only under conditions such as anaerobiosis, peat formation, or the initial colonization of a denuded landscape that plant carbon accumulates faster than it is decomposed (Ghilarov, 1967). In other environments, decomposition more or less balances the primary productivity of the higher plants and algae (Delwiche, 1965; Kononova, 1966; Burges, 1967).

The contribution of plant residues and soil humus to plant growth, soil productivity, and genesis is well recognized (Russell, 1961; Tisdale and Nelson, 1966). Energy flow through producers, consumers, and decomposers is being stressed in ecosystem research (ICSU, 1967). This, plus the questions concerning the geocycle of nutrients in nature (Delwiche, 1965) and the detoxification of environmental pollutants (Breth and Stelly, 1966), makes it imperative that information be obtained relative to the turnover rate of plant constituents, and the recalcitrant humic components in soil.

In this review, no attempt has been made to cover the extensive

organic matter literature. The characterization of plant and soil components relative to their turnover in nature has been stressed.

MAJOR COMPONENTS OF SOIL ORGANIC MATTER

Soil organic matter is a diverse mixture that includes living plant roots, the soil biomass, and partially degraded plant, animal, and microbial components. The latter are intimately associated with the mineral components of the soil and are fairly resistant to further degradation (Mortensen and Himes, 1964; Kononova, 1966). The recognizable plant and microbial components constitute 15 to 25 percent of the total soil organic carbon. These include the carbohydrate and lipid fractions; and a portion of the organic sulfur, phosphorus, and nitrogenous constituents which have been little altered in the soil (Russell, 1961; Stewart et al., 1963; McLaren and Peterson, 1967).

Carbohydrates in Soil

Hydrolyses of the soil with strong acid followed by colorimetric analysis has shown that the carbohydrates usually comprise 5 to 15 percent of the total soil carbon. There is degradation during the preparative procedures. These estimates, therefore, will be minimal if correction factors are not applied (Brink et al., 1960; Gupta, 1967).

Glucose constitutes one-third of the carbohydrate-C in mineral horizons and one-half in forest litters which have not undergone extensive degradation (Table 1). The relative concentration of the sugar monomers mannose, arabinose, xylose, fucose, ribose, and rhamnose, and the presence of 2-O-methylrhamnose and 4-O-methylgalactose in soil indicates their microbial origin (Whistler and Kirby, 1956; Duff, 1961). Except for the forest litter layers and peaty soils, cellulose does not constitute a significant portion of the carbohydrate carbon (Gupta and Sowden, 1964; Schnitzer and Hoffman, 1967).

Keefer and Mortensen (1963) added ^{14}C-labeled glucose and ^{14}C-labeled alfalfa to soil, and followed the incorporation of the radioactivity into the various soil sugar constituents. When alfalfa

TABLE 1

Distribution of Sugars in Hydrolysate of Organic Matter
(Gupta, Sowden, and Stobbe, 1963)

	Podzol		Chernozemic		Gleysolic
	Ao	B	Ah	B	Ap
	Percent of total sugars				
Galactose	15	16	14	15	15
Glucose	54	35	36	34	31
Mannose	15	16	16	14	15
Arabinose	5	9	15	12	15
Xylose	4	9	8	8	11
Fucose-Ribose	3	6	3	5	5
Rhamnose	4	9	8	12	8
Total sugar mg/g OM	161	80	95	68	156
OM mg/g soil	730	140	62	20	24

was added, although all sugars became labeled with ^{14}C, the glucose, galactose, and mannose fractions had a higher specific activity indicating some direct incorporation of sugars. Sixty percent of added glucose was rapidly metabolized into CO_2. The remainder underwent degradation and resynthesis, with the ^{14}C from the number 1 position of glucose being rapidly transferred to other positions in glucose and to other soil sugar monomers.

Although plant carbohydrates are relatively rapidly metabolized by the microbial population, the carbohydrate by-products of microbial reactions can be protected from degradation by four mechanisms:

1. The components of the soil biomass can contribute substantially to the carbohydrates measured by hydrolytic procedures. The mass of living organisms, determined by microscopic methods, often is large and can amount to thousands of pounds per acre. This material is relatively resistant to microbial attack and has a very low metabolic rate (Clark, 1967).
2. Adsorption to bentonite clays has been found to result in attenuated breakdown (Lynch and Cotnoir, 1956).
3. The complexing of sugar constituents with metal ions is another form of stabilization. Martin et al. (1966) found that the fructosan from *B. subtilis* was not protected by complexing with various cations. The *Arthrobacter viscosus* polysaccharide (Fig. 1) containing glucose, galactose, and mannuronic acid (Gasdorf et al., 1965) showed a lag period before attack and resisted degradation for

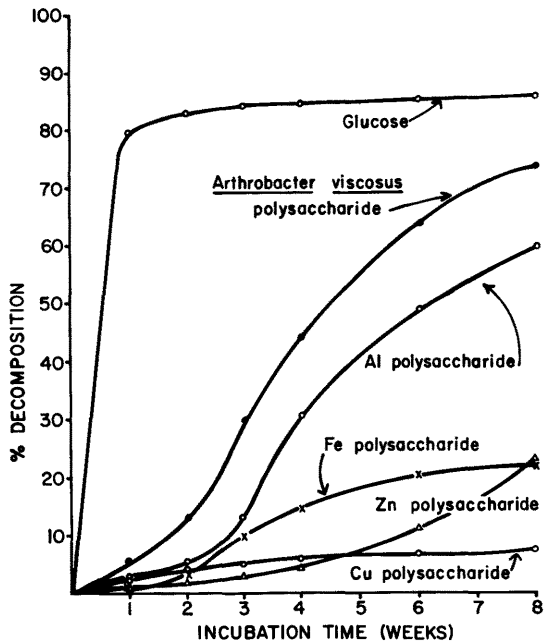

Fig. 1. Decomposition of A. *viscosus* polysaccharide and its Fe, Al, Cu, and Zn complexes in Greenfield sandy loam. (From Martin, Ervin, and Sheppard, 1966. Proc. Sci. Soc. Amer., 30: 196-200.)

considerable periods. The cations such as copper which show microbial toxicity resulted in the greatest protection. However, the complexing of copper with other sugar constituents such as the fructosan from *B. subtilis* did not afford protection indicating other mechanisms also were operative.

4. The condensation of carbonyl groups with amino derivatives by mechanisms similar to those proposed for browning processes in natural products also can explain the stability of some of these materials in soil (Stevenson, 1960; Kononova, 1966).

Only small concentrations of sugars are found in the typical humic acid fraction, the majority being either water soluble or in the humin fraction (Acton et al., 1963; Dormaar, 1967). The determination of sugar monomers in soil hydrolysates has been useful in determining the total amount of materials present but has not indicated their nature (Thomas et al., 1967). Somewhat less than one-third of the carbohydrate carbon of soil is soluble in hot water or dilute buffers (Forsyth, 1947; Bernier, 1958). Polysaccha-

rides extracted from soil with hot water and purified by precipitation, dialyses, and deproteinization contained 39.6 percent carbon, 45 percent oxygen, 1.8 percent nitrogen, 7.1 percent hydrogen, 1.5 percent ash, 3.9 percent carboxyl, and 3 percent methoxyl groups.

The five fractions obtained on a column of diethyaminocellulose all contained glucose, galactose, xylose, arabinose, mannose, fucose, rhamnose, galactosamine, glucosamine, N-acetyl glucosamine, and uronic acid. Their electrophoretic mobility, as determined by free boundary electrophoresis, was related to the uronic content of each fraction (Thomas et al., 1967). The known plant and microbial polysaccharides, however, contain no more than 5 monomers (Smith and Montgomery, 1959; Barker et al., 1965).

The bacterial polysaccharides and carbohydrate fractions are largely responsible for the stabilization of soil structure (Harris et al., 1964). The amount of carbohydrates produced and its aggregating ability, relative to the available substrate and nutrients such as nitrogen and phosphorus, has been investigated in some detail, as have some of the mechanisms involved (Chesters et al., 1957; Acton et al., 1963; Harris et al., 1966). The specific chemical composition and structures of these materials in soil, however, are still unknown.

Phosphorus Compounds

The known groups of phosphorus compounds in soil include the inositol phosphates, nucleic acid derivatives, sugar phosphates, phosphoproteins, and phospholipids (McKercher, 1968). Extractions of soil with ethanol, ether, acetone, and chloroform have indicated that 3 to 7 μg/g of phosphate are extractable as a phospholipid. Although phosphoproteins and sugar phosphates, especially glucose-1-phosphate, exist in soil, their relative content is low also and probably is associated with the microbial biomass.

The nucleic acid phosphorus usually accounts for 5 to 10 percent of the total organic soil-P (Anderson, 1967). Soil nucleic acid derivatives have been extracted from soil with dilute alkali, and analysed by elution chromatography and absorption spectrophotometry following acid hydrolysis (Adams et al., 1954). The proportion in which the basic derivatives of the nucleic acid (cytosine>guanine>thymine>adenine) occur indicates polynucleotides derived from DNA, the amount being greater than could

be accounted for in undecomposed microbial cells (Anderson, 1958). The material is largely of microbial origin as shown by the predominance of guanine, and cytosine, and the absence of 5-methylcytosine which replaces a considerable portion of the cytosine in DNA from plant sources (Anderson, 1967).

Although inositol itself is widespread in nature, the presence in soil of the polyphosphates of five stereoisomers, myo-, scyllo-, neo-, and the racemic dl forms, differentiate the soil polyphosphates from other sources. Naturally occurring neoinositol has been identified only from soil sources. Myoinositol is the only phosphorylated inositol which appears to occur naturally in sources other than soil (Cosgrove, 1966; McKercher, 1968). The penta- and hexaphosphates usually comprise 15 to 45 percent of the total organic phosphorus with the myo- plus dl- forms being present in a ratio of 1 to 3 times that of the scyllo- forms. The hexaphosphates have been shown by McKercher and Anderson (1968b) to be about 3 times as abundant as the pentaphosphates. Some soils, however, appear to have undetectable amounts of inositol phosphates (Cosgrove, 1966).

There is no evidence to indicate that the organic soil phosphate, which constitutes 30 to 70 percent of the total phosphate, can be utilized to any great extent by plants. It must be mineralized before it is in an available form (Cosgrove, 1967). Organisms capable of dephosphorylating all known organic phosphates of plant origin have been isolated from soil (Szember, 1960; Greaves et al., 1963).

Enwezor (1966, 1967) found upon incubation of soil that the mineralized phosphorus was closely correlated with the organic phosphorus expressed as a percentage of the total phosphorus in the soil, but not with the carbon: organic phosphorus ratio. Although it is known that increased soil temperature (van Diest and Black, 1959) and alternate wetting and drying (Birch and Friend, 1961) result in higher mineralization rates, a close relationship between the mineralization of phosphorus and that of carbon (Thompson et al., 1954; Wier and Black, 1968) has not been found. Soils having a high nitrogen content and a low C:N ratio usually can be counted on to release significant concentrations of available nitrogen during crop growth (Harmsen, 1964; Bartholomew and Clark, 1965). However, some of the agricultural soils containing the largest known concentration of organic phosphorus (McKercher and Anderson, 1968a) are amongst the most deficient in plant available phosphorus (Rennie and Clayton, 1966).

Soil Organic Sulfur

Sulfur exists in a wide range of forms in plants, animals, and microorganisms. This includes such diverse forms as amino acids, sulfonium compounds, sulfate esters, sulfides, sulfoxides, isothiocyanates, and miscellaneous compounds such as lipoic acid, vitamins, and antibiotics (Freney, 1967). The organic form of sulfur dominates in soils that undergo leaching, with inorganic forms being present also in the more arid regions (Williams and Steinbergs, 1962).

The organic soil sulfur is usually divided into two major fractions, the sulfate esters and carbon bonded sulfur. The sulfate esters can be reduced to hydrogen sulfides with hydroiodic acids and are readily hydrolyzed to inorganic sulfate by acid or alkali. This sulfur may exist as sulfated polysaccharide, phenolic sulfates, choline sulfates, and sulfolipids. The sulfate esters appear to comprise slightly more than one-half the total sulfur of organic soils in Quebec and in the mineral soils of Australia. The carbon bonded sulfur, e.g. cystine, cysteine, and methionine accounts for 40 percent, and inorganic and absorbed sulfur (Freney et al., 1962; Lowe, 1964; Freney, 1967) the remainder.

The distribution of sulfur in some Canadian Chernozemic and Podzolic soils is shown in Table 2. Sulfate esters, reducible by hydroiodic acid, accounted for two-thirds of the total sulfur present in the Chernozemic Ah horizon. Except for the Grey Wooded Lh layer, which was composed primarily of undecomposed materials,

TABLE 2

Sulfur Fractions in the Upper Horizons of Selected Alberta Soils[a]

Soil Type	Horizon	Total S	Easily soluble sulfate	Adsorbed sulfate	HI-S[b]	C-S[b]	HI-S+ C-S as percent total S
		μg/g	μg/g	μg/g	μg/g	μg/g	
Chernozemic	Ah	435	18	18	269	74	80
Chernozemic	Bm	273	24	13	197	22	80
Grey Wooded	Lh	700	39	16	218	190	59
Grey Wooded	Bt	80	5	7	37	12	63

[a] From Lowe, 1965. Canad. J. Soil., 45:297-303.
[b] HI-S, HI-reducible sulfur; C-S, carbon-bonded sulfur.

the sulfate esters were present in concentrations at least three times that of the carbon bonded sulfur. Sulfated polysaccharides were isolated from all surface horizons, but in no case accounted for more than 2 percent of the total sulfur. An inert sulfur fraction resistant to strong oxidizing and reducing agents has been detected in residues of samples subjected to exhaustive extractions by acid and alkali (Lowe, 1964, 1965).

Nelson (1964) found a close relationship between sulfur mineralized from soil organic matter during a six-month incubation period, and the organic-sulfur content of the soil. During a six-month incubation period, 7.5 percent of the carbon, 3.5 percent of the nitrogen, and 6.0 percent of the sulfur were mineralized. This soil had a carbon:nitrogen:sulfur ratio of 126:10:1.1 which is close to the normally assumed ratio of 100:8:1:1.2 for the C:N:S:organic-P ratio (Eaton, 1922; Walker, 1957). There is evidence to indicate that the mineralization of organic sulfur is more dependent on the recently added sulfur-containing residues than the sulfur content of the total soil organic matter (Barrow, 1960, 1961).

Scharpenseel and Krausse (1963) followed the incorporation of labeled sulfate, methionine, and cystine into soil. Column-chromatographic fractionation following hydrolysis of the treated sample showed that the S-amino acids occurring in soil humic materials are mainly cysteic acid, cysteine-sulfinic acid, taurine, methionine sulfone, cystine, and methionine. The individual amino acids were metabolized in the soil without incorporation into humic constituents. Addition of labeled plant residues, however, resulted in the incorporation of ^{35}S into the humic acids.

Soil Humic Components

The polydisperse, multi-component, humic system having acidic functional groups and relatively high molecular weights (van Dijk, 1966) never occurs in pure form, but is intimately associated with other organic components and complexed with the mineral soil colloids (Scheffer and Ulrich, 1960; Kononova, 1966; Scharpenseel, 1966). The major difficulty in its study has been the inherent problem of obtaining meaningful extractants (Dubach and Mehta, 1963; Dubach et al., 1964; Mortensen and Himes, 1964).

It is recognized that humic materials, comprising 50 to 85 percent of the total soil organic matter, show a great deal of

similarity even though isolated from many different locations (Hansen and Schnitzer, 1966; Kononova, 1966). The organic matter from Podzol B horizons has been characterized extensively (Jakab et al., 1961; Dubach and Mehta, 1963; Schnitzer, 1965; Schnitzer and Skinner, 1965; Hansen and Schnitzer, 1966). The techniques developed for extraction, functional group analysis, molecular weight distribution, degradation analysis, and other characteristics such as infrared and x-ray spectra are proving very useful. The extrapolation of data relative to the chemical characteristics and dynamics of materials from the Podzol B horizons to other soils must, however, be done with caution. The humic materials from the Podzol B are illuvial in nature, resulting from the leaching of low molecular weight substances from the surface and deposition in the lower horizon. In most other soils, the majority of organic matter is formed in situ from the microbial degradation of the litter and roots. At the surface it is influenced by plants, microbial growth, wetting and drying, cultivation, and the presence of stabilizing clays and cations.

Heteropolycondensate is the term used for the condensation products of aromatic compounds combined with products of protein decomposition and the possible participation of carbohydrate materials. The term was first proposed by Dragunov in 1948 (Kononova, 1966). His scheme for the structure of humic materials agrees in principle with the ideas of many contemporary investigators. The more widely referenced diagram of Flaig (Flaig, 1960; Steelink and Tollin, 1967) is very similar. Both schemes assume that the linkages between structural units and monomers are established primarily by covalent bonds, e.g., ether linkages, -NH-, -S-, and $-CH_2-$ bonds. The specific structures involved and the exact relations between the protein-aceous and aromatic portions are, however, unknown and a number of other possible structures have been proposed (Scheffer and Ulrich, 1960; Felbeck, 1965; Finckle, 1965).

The formation of humic materials is not enzymatically controlled, but is the result of condensations of microbial degradation products of a wide array of phytochemicals (Flaig, 1964, 1966). Therefore, the physical and chemical characteristics of the environment, at the time of formation, greatly affect their structure. These materials, although having closely related structures, cannot be expected to be uniform, and their characteristics must be related

back to their environment, both in terms of soil genesis and the plant ecosystem.

The characterization of the non-nitrogeneous moiety of humic material has usually involved stepwise degradation of the substances. Hoppe-Seyler (1889) was one of the first to show the presence of the aromatic ring. He fused peat with concentrated KOH, and detected pyrocatechol and protocatechuic acid among the fission products. Alkaline hydrolysis, acid hydrolysis, or oxidation has generally resulted in a yield of 2 to 6 percent of the humic carbon as identifiable compounds (Schnitzer and Wright, 1960; Greene and Steelink, 1962; Jakab et al., 1963; Schnitzer and Desjardins, 1964).

Whereas lignin is completely degraded by HNO_3, up to 50 percent of the organic fraction of most soils is resistant to HNO_3 oxidations (Felbeck, 1965). The aromaticity of the organic matter of Podzol soil has been investigated by heating the material in air at 170°C for approximately 500 hours (Wright and Schnitzer, 1961). It was concluded that half the organic material is present in aromatic compounds. Hydrogenation techniques when applied to soil humic materials have not been as successful as similar studies with lignin (Felbeck, 1965). Somewhat less than 2 percent of the total carbon was isolated as *n*-C25 or *n*-C26 hydrocarbons with other unknown products of hydrogenation representing approximately 63 percent of the carbon in the non-hydrolysable fraction.

Paper, thin layer, and gas chromatography have been used to separate the products obtained from soil humic acids by reductive cleavage with sodium amalgam. Burges et al. (1964) recovered up to 30 percent of humic acid carbon of Podzolic material as ether soluble substances consisting of a complex of phenolic and phenolic acid monomers. The contribution of lignin from the overlying vegetation to the Podzol B humic acid was demonstrated by the presence of relatively unchanged lignin residues. The compounds for which identification was claimed were *p*-hydroxybenzoic acid, vanillic acid, syringic acid, protocatechuic acid, guaiacylpropionic acid, syringylpropionic acid, and 3,5-dihydroxybenzoic acid (Burges et al., 1964). A similar study on a Brunizemic soil showed the presence of at least 8 components, the majority of which occurred in compounds other than phenols and phenolic acids. Vanillic acid and syringic acids were positively identified, and strong evidence was obtained for the occurrence of vanillin and syringaldehyde. Units

atypical of lignins were not found (Mendez and Stevenson, 1966; Stevenson and Mendez, 1967).

Spectrographic methods are some of the most useful nondestructive techniques for examining unknown compounds. The ultraviolet and visible spectra of soil humic materials are rather featureless (Ziechman, 1964; Kleist and Mucke, 1966). However, the relative absorption of light in the visible range can characterize humic material. This has been designated as the E_4:E_6 ratio or the Δlog k of a plot of the logarithm of absorption versus wavelength (Kumada, 1959; Scheffer and Ulrich, 1960; Kononova, 1966; Kuroda, 1966). The E_4:E_6 ratio is closely correlated to the soil color characteristics, especially *value*, determined by spectrophotometric measurements over the visible range (Shields et al., 1968). It also is related to the degree of condensation of the aromatic nuclei (Kononova, 1966). In addition, there appears to be a close correlation of the mean residence time of humic fractions within a soil with the E_4:E_6 ratio of those fractions (Campbell et al., 1967b).

Figure 2 shows the change in infrared spectra during the rotting of lignin in soil, and some typical humic acid spectra. Infrared spectroscopy, especially when used in conjunction with chemical derivative studies, has indicated the functional groups and aromaticity of the organic matter (Broadbent and Bradford, 1952; Dubach and Mehta, 1963; Mortensen and Himes, 1964; Schnitzer and Gupta, 1965; van Dijk, 1966). Because of the complexity of soil organic matter, infrared spectroscopy has had only limited application relative to the structural features of soil humic compounds.

Published values for the molecular weight of humic acids range from 3,000 to >100,000 with the average molecular weight derived from osmotic and cryoscopic measurements always being smaller than that obtained by viscosity determinations (van Dijk, 1966). Computing molecular weights from sedimentation velocities and diffusion in the ultracentrifuge usually yields a value between the two (Schnitzer and Desjardins, 1962; Orlov and Gorshkova, 1965). Similar values have been obtained with Sephadex gel chromatography (Ferrari and Dell'Agnola, 1963; Mehta et al., 1963; de Borger, 1967). This technique works well with material such as fulvic acids and mobile humates, especially if electrolytes are used for elution (Posner, 1963; Mathur and Paul, 1967b). The highly aromatic nonmobile humic materials are, however, adsorbed to the gel and cannot be quantitatively removed (Atherton et al., 1967; Biederbeck and Paul, 1968).

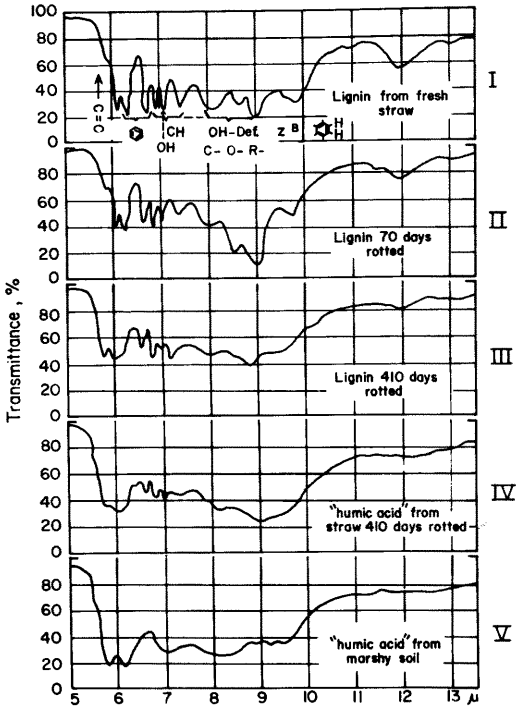

Fig. 2. Infrared spectra of lignin fractions. I, Bjorkman lignin from fresh straw. II, Bjorkman lignin after 70 days of incubation. III, Bjorkman lignin after 410 days incubation. IV, Humic acid from moor soil extracted with a mixture of 0.1 N NaOH + 0.2 N NaF. (From Flaig, 1966. Courtesy of Pergamon Press, Inc.)

Flaig and Beutelspacher (1954), on the basis of viscosity and electron microscopy data, concluded that humate particles of 60,000 molecular weight were spheroidal with a diameter of 60 to 80 A. Other data indicate that, although the lower molecular weight materials are spheroidal, the higher molecular weight humates are ellipsoidal in nature (Piret et al., 1960; Wershaw et al., 1967). By necessity, measurements such as this utilize suspended materials. The shape of the humates in the soil may well be different.

The postulation (Scheffer et al., 1958) that free radicals were involved in the synthesis of humic materials has since been confirmed by a number of workers (Rex, 1960; Green and Steelink, 1962; Steelink and Tollin, 1962; Lagercrantz and Yhland, 1963). The relatively high concentration of stable free radicals in the free state (Table 3) and the reversible enhancement of spin concentra-

TABLE 3

Spin Concentrations of Various Lignin Preparations and Humic Acids[a]

Sample	Spins/g	Molecular weight	Spins/mole
Lignins			
BNL,[b] spruce	5×10^{16}	1,000+	5×10^{19}
Norway spruce sulfuric acid	3×10^{16}	5,000+	1.5×10^{20}
Yellow pine, Kraft	3×10^{17}	7,000+	2.1×10^{21}
Calcium lignin sulfonic acid	3×10^{17}	10,000+	3×10^{21}
Humic aids			
Fulvic acid from Wisconsin podzol	3×10^{17}	–	–
Humic acid from Wisconsin podzol	0.8×10^{18}	–	–
Humic acid from muck soil	0.3×10^{18}	–	–
Humic acid from California podzol	2×10^{18}	20,000+	4×10^{22}
Humic acid from English podzol	1.4×10^{18}	20,000+	3×10^{22}
Humic acid from Arizona brown-forest soil	0.8×10^{18}	–	–

[a] From Steelink and Tollin, 1962. Biochim. Biophys. Acta, 59:25-34.
[b] Brauns' native lignin (BNL)

tion upon basification indicate the presence of quinone structures (Atherton et al., 1967; Steelink and Tollin, 1967). Free radicals could occur in structures such as those envisioned by Dragunov (Kononova, 1966) and Flaig (1960). These structures assume the presence of individual aromatic rings. The occurrence of free radicals can also be explained by a polycyclic, aromatic core surrounded by carbohydrates, polypeptides, relatively simple phenols, and metals (Scheffer and Kickuth, 1961; Cheshire et al., 1967; Scheffer and Ziechman, 1967).

There is still a difference of opinion concerning the occurrence of the polycyclic aromatic core in soil humates (Kumada et al., 1961; Savage and Stevenson, 1961; Cheshire et al., 1967; Hansen and Schnitzer, 1966) and the nature of the carbonyl group. Schnitzer and Skinner (1965), Wagner and Stevenson (1965), and Theng and Posner (1968) were not able to demonstrate the occurrence of quinones in their soil humic materials. These differences must be resolved because the present theories concerning the formation of these materials are based on their quinonoid and aromatic nature. Since both polycyclic and individual aromatic structures can give rise to stable free radicals, their occurrence in a specific soil will depend on the nature of the vegetation, the chemical characteristics of the soil, and the microbial population present.

The Nitrogen Component

Nitrogen is intimately associated with carbon, hydrogen, sulfur, and oxygen in the skeleton of the humic moiety. Hydrolysis with 6N HCl followed by ion-exchange chromatography has indicated that the following amino acids always occur: phenylalanine, leucine, isoleucine, valine, alanine, glycine, serine, threonine, aspartic acid, butyric acid, arginine, histidine, lysine, proline, hydroxyproline, α-NH_2-butyric acid, β-alanine, and tyrosine (Stevenson, 1956; Bremner, 1965a). The detection of non-protein amino acids that are often of microbial origin was not unexpected: α-ε-diamino-butyric acid, ornithine, and 3,4-dihydroxyphenylalanine have been found in soil hydrolysates. Most of these also occur as free amino acids extractable from soil in small concentrations by mild treatment (Paul and Schmidt, 1961; Paul and Tu, 1965).

The general distribution of the major forms of nitrogen in a number of soils is shown in Table 4. The acid-insoluble nitrogen which accounts for approximately one-fifth of the total nitrogen (Ferguson and Sowden, 1966) is considered to be cyclic. It can also be the nitrogen of an amino acid attached directly to an aromatic ring by a covalent bond. The hexosamine nitrogen found in soil hydrolysates is composed primarily of glucosamine and galactosamine. Approximately 20 percent of the soil nitrogen appears in the hydrolysate in a soluble form other than hexosamine or ammonia nitrogen (Bremner, 1965a).

TABLE 4

Percentage of Total Soil Nitrogen in Various Fractions Following Acid Hydrolysis[a]

Great soil group	Total soil N (percent N)	Acid-insol. N	α-amino N	Total amino N	Hexo-samine N	Am-monia N	Uniden-tified
Black Chernozemic	.37	21.6	24.1	29.7	3.0	19.2	26.5
Black Chernozemic	.21	19.0	34.3	41.9	6.2	26.2	6.7
Black Chernozemic	.16	18.7	24.4	30.0	2.5	22.5	26.3
Podzol	.93	14.9	36.0	44.3	2.7	18.5	20.5
Brown Forest	.58	25.9	30.9	38.8	3.3	18.3	13.7

[a] From Ferguson and Sowden, 1966. Canad. J. Soil Sci., 46:1-6.

Studies on the more soluble complexes of soil organic matter indicate that the nitrogeneous constituents associated with the fulvic fractions of organic matter occur in compounds of non-protein nature. However, one-quarter of the fulvic nitrogen could be liberated as α-amino acids upon hydrolysis and a considerable portion of the fulvic nitrogen was readily deaminated during acid hydrolysis (Forsyth, 1947). Stevenson (1960) postulated that the fulvic materials separated on charcoal columns represented the condensation of carbonyl and amino derivatives by mechanisms similar to the browning reaction.

The lignoprotein concept of humic materials postulated that humic acids are formed by a reaction between lignin and protein materials and that humic nitrogen is largely protein. The humic materials are relatively low in molecular weight and only one-third of the total nitrogen of most humic preparations can be accounted for as protein nitrogen. This has led to the presently held theory that amino acids formed either by proteolysis or synthesis by microorganisms react with polyphenols from oxidized lignin decomposition products to form soil humic acids (Laatsch et al., 1952; Flaig, 1960; Kononova, 1966).

Swaby and Ladd (1962) and Flaig et al. (1963) suggested that the amino acids are condensed through the amino groups with quinones leaving free carboxyl groups. The diamino acid lysine and thiol-containing cystine would crosslink with neighboring quinones to form a three dimensional copolymer consisting of many different amino acid, phenol and quinone groups without ordered sequence. The resistance of humic acids to microbial and chemical decomposition could then be explained by steric hindrance. A large spherical molecule consisting of many heterogeneous units, irregularly crosslinked by different covalent bonds, would require innumerable extracellular enzymes from many different microorganisms to dissemble it piece by piece from the outer surface. This theory takes into account that humus molecules are most rapidly poly-condensed or polymerized from free radicals formed enzymatically in high concentrations of monomeric substances. The polyphenols can be of microbial origin as well as from lignin (Flaig et al., 1963; Kang and Felbeck, 1965; Haider and Martin, 1967).

The early work of Hobson and Page (1932), which indicated that at least a portion of the nitrogen of humic materials is susceptible to hydrolysis with trypsin, has recently been repeated with papain (Scharpenseel and Krausse, 1962) and with pronase

(Ladd and Brisbane, 1967). The liberation of amino acids by pronase, accounting for 27.6 percent to 39.4 percent of those released by hot acid hydrolysis, is further evidence for the occurrence of peptide bonds in soil humic acid.

Ladd and Butler (1966) prepared synthetic, phenolic polymers soluble in 0.5 N NaOH and precipitable by HCl at pH 1 by reacting either catechol oxidized by silver oxide or *p*-benzoquinone with single amino acids, mixtures of amino acid, simple peptides, or with proteins at pH 8.0 and 45°C (Table 5). Comparing the percentages of amino-acid N released after acid hydrolysis shows that the synthetic polymers in which amino acids are incorporated as peptides or proteins more closely resemble natural humic acids than those prepared utilizing individual amino acids. It seems reasonable that if humic materials are in part formed by the polymerization of various phenols and quinones, then opportunities will exist for the incorporation of amino acids, peptides, and proteins.

The Sevag technique for removing proteins from agar solutions when applied to sodium pyrophosphate extracts of soil and com-

TABLE 5

Analyses of Synthetic Polymers and Humic Acids[a]

Polymer or humic acid	Polyer-N (per-cent)	Acid-Soluble-N (per-cent)	Amino-N (per-cent)	Ammo-nia-N (per-cent)	Acid hydrolysed polymer (per-cent)	Resi-due-N (per-(cent)
Catechol/-	0.00	0.0	0.00	0.0	5.2	0.00
Catechol/glycine	4.46	25.4	10.6	9.3	13.2	3.95
Catechol/casein hydrolysate	3.51	28.9	11.1	8.9	10.8	2.83
Catechol/diglycyl-glycine	3.35	65.4	32.9	7.0	15.8	1.86
Catechol/protamine	4.13	81.5	46.9	0.0	23.8	0.83
Benzoquinone/ digylcyl-glycine	6.15	65.7	31.1	5.3	25.9	2.95
Benzoquinone/ protamine	3.68	77.0	39.1	0.8	23.6	0.96
Benzoquinone/casein	5.55	93.0	70.9	10.9	42.1	1.22
Humic acid (from peat)	2.09	62.5	50.9	10.2	26.9	1.01
Humic acid (from loam)	3.46	60.0	35.8	8.4	34.5	1.94

[a] From Ladd and Butler, 1966. Courtesy of Pergamon Press, Inc.

posts has resulted in the isolation of a small portion of the organic material as a lignoprotein or a lignopeptide (Jenkinson and Tinsley, 1959). Simonart et al. (1967), using phenols as a protein solvent, isolated a humoprotein containing 11 percent nitrogen. A nondialysable protein fraction containing 14.8 percent nitrogen was obtained by further purification using paper chromatography in a phenolic system. This initial isolation of a protein from humus in reasonable quantities has been confirmed by Biederbeck and Paul (1968) who fractionated humic acids with phenol and subsequently purified the material in the phenolic phase using molecular screens and the phenol complexing agent polyvinylpyrrolidone (PVP). A high molecular weight, proteinaceous material was obtained by using the solvation characteristics of phenol and its ability to dissociate hydrogen bonds, in conjunction with the phenolic scavenging properties of PVP (Westphal and Jann, 1965; Loomis, 1968).

MICROBIOLOGICAL TRANSFORMATIONS IN SOIL

Although resistant to microbial attack, soil humus is not immune to biodegradation (Birch and Friend, 1961). The organisms involved represent a wide range of different types, i.e., *Penicillium, Aspergillus* (Kudrina, 1951), *Polystictus* (Burges and Latter, 1960), *Trichoderma, Streptomyces, Nocardia* (Schonwalder, 1958), *Proactinomyces, Actinomyces* (Ochilova, 1961a, b), *Arthrobacter* (Stevenson, 1967), *Pseudomonas* (Nikitin, 1960), *Corynebacterium* and *Bacillus* (Schonwalder, 1958), and sulfate-reducing bacteria (Alexandrova, 1953). It has generally been observed that these organisms can utilize the nitrogeneous side chains of the humus compounds provided the substrate contains a readily utilizable substrate. Soil suspensions and mixed cultures of organisms bring about a greater degree of degradation than individual pure cultures (Pontovich, 1938; Volkova, 1961), and sterilization or alkalization of humic substances greatly increases their vulnerability to microbial attack.

The usefulness of chemical degradation techniques is limited because reactions which break the linkage between monomers are usually strong enough to corrode or alter the monomers they set free. Enzymes are specific and their reaction conditions mild. The

initial products of microbial enzymatic cleavage should, therefore, accurately reflect the constituents and structure of humic acids and prove a useful complement to chemical techniques.

The peroxidase activity of microbial cultures has been directly related to their ability to decompose humic material (Mishustin and Nikitin, 1961). Hurst (1963) found that carboxyl groups of humic acids were reduced during partial decomposition by *Polystictus versicolor*, and concluded that although decomposition of humic acid is primarily an oxidation process the initial step in biological degradation of humic acids is reduction. This step was simulated by sodium amalgam in subsequent chemical investigations (Hurst et al., 1961; Burges et al., 1963).

A strain of *Penicillium frequentans* has been found to attack a broad spectrum of humic materials, with different molecular weights, obtained from a Chernozemic soil (Mathur and Paul, 1967a), with the smallest fraction being degraded to the greatest extent (Table 6). Refiltration of the >35,000 molecular weight range through Sephadex G-100 showed that the larger moieties of humic acid were not completely degraded during the attack but were reduced to intermediate- or small-sized molecules. Hydrolysis of the ether bond present in humic acid appeared to be the major method of degradation (Paul and Mathur, 1967). Salicyl alcohol and salicylaldehyde were measured after fungal cleavage under conditions of restricted aeration. Salicyl alcohol may be produced by the reduction of salicylic acid through salicylaldehydes by several fungi which inhabit the soil (Hurst, 1963). These components have also been found in the degradation of polyaromatic compounds such as naphthalene (Bhat et al., 1959). The *Penicillium* used in this investigation, however, did not reduce salicylic acid in either aer-

TABLE 6

Molecular Size Distribution of Humic Acids and Extent of Degradation of Various Size Fractions

Molecular weight range (ooo)	Percent of original total humic acids	Percent degraded
>35	21.0	40.0
8 to 35	30.0	34.0
6 to 8	22.0	35.0
<6	27.0	53.0

ated cultures or in low oxygen tension cultures. Nor did it appear to utilize naphthalene (Mathur and Paul, 1967a, b).

A proposed scheme for the degradation of plant residues and the formation of soil organic matter is shown in Figure 3. The carbohydrate and proteinaceous plant constituents serve as substrates for energy and growth of microorganisms. The microorganisms in turn synthesize amino acids, proteins, cell wall constituents, and carbohydrates. These products can persist in the soil for extended periods as a portion of the living soil biomass. In addition, plant carbohydrates, proteins, and the microbial cell structures can be protected by colloid adsorption (Ensminger and Gieseking, 1942; Esterman et al., 1959).

The higher molecular weight lignin undergoes oxidative degradation followed by demethylation and removal of side chains. The lignin decomposition products themselves can polymerize by cross-linking between side chains and between rings. Microbial attack of the decomposition products results in a further decrease of the carbon, and an increase in the oxygen content. Nitrogen is incorporated into the molecule by condensation reactions (Whitehead and Tinsley, 1963; Flaig, 1966).

The amorphous, nonspecific polycondensates constituting humic materials require a rich complement of different enzymes to bring

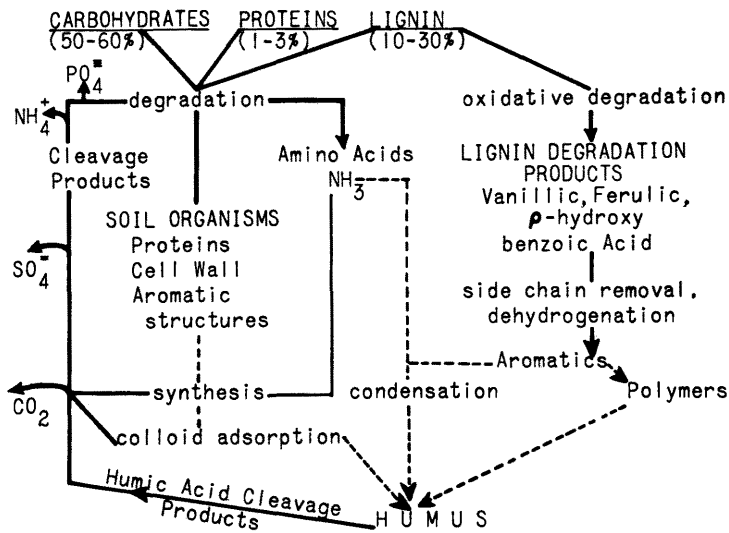

Fig. 3. Degradation of plant residues and formation of soil organic matter.

about total dismemberment. A single organism cannot be expected to have all the enzymes needed to accomplish this task. It is often noted, especially in soils which do not have a high organic matter content, that the addition of fresh organic residues enhances the degradation of the native material (Broadbent and Norman, 1946; Chahal and Wagner, 1965). This priming effect can be explained in part by the fact that the addition of available substrate greatly increases the concentration of extracellular enzymes available for decomposition of the nonsoluble humic material. In the presence of added substrate, there is some channelling of the added carbon and nitrogen into soil humic compounds and the fairly large biomass. The full significance of these interchanges is as yet unknown. The internal cycling of components in soil must, however, be taken into account when utilizing mathematical models to describe the turnover of a specific substrate (Jenkinson, 1966a; Clark, 1967).

THE DYNAMICS OF THE DEGRADATION OF PLANT COMPONENTS AND SOIL HUMUS

The application of tracer techniques (^{14}C, ^{15}N) has made it possible to follow the fate of phytochemicals in soil. A uniform labeling of the plant material is a prerequisite when unfractionated labeled plants are used in the study of the transformation of soil organic matter. Sauerbeck and Führ (1966), and Pinck and Allison (1951), summarizing nonisotopic work on the decomposition of plant residues in soil, concluded that the percentage of added carbon released when plant material was incubated with soil is nearly always independent of the quantity added, provided the carbon addition does not exceed 1.5 percent of the dry weight of the soil and the decomposition is allowed to continue for at least 3 to 6 months. More recent work with labeled plant material (Stotsky and Mortensen, 1958; Jenkinson, 1966a) supports those findings. If adequate nitrogen is present, the amount of carbon lost is directly proportional to the amount added. Nonlinear relationships are usually found when the soil supply of mineral nutrients is limiting.

The data from a number of incubation experiments with ^{14}C-labeled materials are shown in Table 7. After a 30-day incubation period, 11 percent of the initially added glucose carbon still remained in the soil. This material was fairly evenly distributed

TABLE 7

Immobilization and Distribution of Organic ^{14}C After Incubation in the Soil of ^{14}C Components

^{14}C components	Amount added (mg C/100 g)	Incubation period days	Immobilized ^{14}C (percent init.)	Percent Distribution of immobilized ^{14}C			References
				Fulvic acid	Humic acid	Humin	
Barley	211	100	50	11.8	25.9	49.4	Sorensen, 1963
Ryegrass	120	90	33	–	–	–	Jenkinson, 1965
Ryegrass	120	365	31	–	–	–	Jenkinson, 1965
Ryegrass	120	1095	21	–	–	–	Jenkinson, 1965
Ryegrass	120	1460	19	–	–	–	Jenkinson, 1965
Proteins (globulin, foliage)	50	30	55	16.3	51.3	18.5	Simonart and Mayaudon, 1961
Cellulose	100	30	20	25.3	29.6	45.2	Mayaudon and Simonart, 1959[b]
Lignin	100	60	70.3	10.3	48.6	40.9	Mayaudon and Simonart, 1959[b]
Syringaldehyde	100	30	17	25.1	41.3	33.4	Mayaudon and Simonart, 1959[b]
Glucose	100	30	11.3	20.2	34.5	45.0	Mayaudon and Simonart, 1959[a]

throughout the fulvic acids, humic acids, and humin. Similar results have been obtained with other carbohydrates such as cellulose and hemicellulose extracted from plant materials grown in a biosynthesis chamber containing ^{14}C (Sorensen, 1967).

Ryegrass tops uniformly labeled with ^{14}C were allowed to decompose for four years in soil under field conditions (Table 7). Two-thirds of the added labeled carbon was evolved as CO_2 during the first 90 days. Thereafter, decomposition was much slower, with one-fifth of the labeled carbon remaining in the soil after four years. Throughout the period, labeled carbon was less resistant to degradation than native soil organic matter. Even in the fourth year, the percentage of labeled carbon decomposing per year was four times that of the unlabeled carbon (Jenkinson, 1965).

The data in Table 7 also indicate that the fractionation techniques yielding fulvic acids, humic acids, and humin, although very useful from a physicochemical standpoint, have limited applicability in describing the dynamics of the organic matter constituents. The fulvic acids contain the protoplasmic constituents of the soil organisms whereas the insoluble cell-wall constituents and the material rapidly adsorbed to the soil colloidal fractions appears in the humin fraction (Zeller et al., 1966; Mayaudon and Simonart, 1963; Mayaudon, 1966).

The agents that extract components with a high specific activity after the addition of radioactive plant materials and subsequent incubation are poor extractants of humified material and tend to keep it flocculated. They are neither strongly hydrolytic nor oxidative. Materials such as benzene and methanol, $Ba(OH)_2$, cold HCl, or boiling water (Chahal et al., 1966; Sorensen, 1967; Jenkinson, 1968) have been found to be most effective in extracting heavily labeled organic carbon from soil. This fraction nearly always has a low C/N ratio and a significant proportion of it is considered to be extracted from the soil biomass.

When readily decomposable substrates such as glucose, hemicellulose, or cellulose decompose in soil, the carbon from these fractions tends to be distributed throughout the soil organic matter components in a pattern which is similar to that of organic nitrogen as opposed to that of organic carbon (Simonart and Mayaudon, 1966; Jenkinson, 1968). Amino acids usually are the most heavily labeled fraction after the addition of ^{14}C-labeled substrates. Sorensen (1967) found that when cellulose and hemicellulose carbon was added to soil followed by incubation, 66 percent of the material was

decomposed during the first 30 days. The recovery of original cellulose carbon as amino acid carbon amounted to 6 to 11 percent after 10 to 30 days, and decreased to about 5 percent after 300 days incubation. After this time, the decomposition proceeded very slowly and almost the same recovery was observed after 600 and 900 days of incubation as after 300. The total residue of the original cellulose carbon recovered as amino acid carbon approximated 30 percent of the radioactivity left in the soil. The addition of mineral nitrogen prior to incubation and the presence of montmorillonite resulted in a stabilization of the added carbon and greater recoveries as amino acid.

The nitrogen immobilized during decomposition of carbonaceous residues is slowly mineralized in subsequent years. Stewart et al. (1963) found that less than one-half of immobilized fertilizer-^{15}N was released for plant uptake during four successive croppings in the greenhouse. Fractionation of the soil-N showed that about 75 percent of the nitrogen utilized by the plant was derived from the acid-hydrolyzable fraction (Stewart et al., 1963; Porter et al., 1964). The acid-hydrolyzable constituents of the soil organic matter can be considered to contain the biological active carbon and nitrogen which is slowly released during subsequent years (Jansson, 1963; Persson, 1966; Legg and Allison, 1967).

MATHEMATICAL MODELS OF PLANT RESIDUE AND SOIL HUMIC TRANSFORMATIONS

A number of long-term field experiments, in which the addition of various components of plant residues and the level of soil organic matter at different times was measured, have been conducted. These experiments indicated that the process of decomposition and accumulation of residues in soil can be represented by exponential functions of the type $y = e^{rt}$. This implies that the process of decomposition can be represented logarithmically so that a fixed multiplication factor (the decomposition factor) determines the loss. The equations have been modified to recognize the fact that different components of soil organic matter decompose at different rates (Woodruff, 1949; Henin et al., 1959) and to take into account seasonal changes (Russell, 1964). On the basis of these functions, various parameters can be calculated: the time it takes for a year's

crop of litter to be reduced to half its weight; or the maximum accumulation or degradation that will occur before equilibrium conditions are established (Olson, 1963; Minderman, 1968). This has made it possible to utilize mathematical models to account, at least in part, for the effect of different management practices, climate, and soil factors on the turnover rate of soil organic matter constituents (Kortleven, 1963; Jenny, 1965).

Minderman (1968), utilizing data from forest soils in which the annual addition of the litter components and the amount of organic matter in the soil had been measured over a long period of time, calculated an overall degradation rate from the knowledge of the degradation rate of the individual components. By summation of the decomposition rates of the individual fractions of the leaf litter, i.e. cellulose, hemicellulose, lignins, etc., it was shown that the calculated decomposition curve corresponded in shape with that actually determined over a period of 15 years. The actual curve under field conditions showed a lower degradation in later years than the summation of the individual components.

It is known that there is an internal cycle of carbon within the soil, with the constituents from materials such as cellulose rapidly entering the amino acid forms (Sorensen, 1967; Jenkinson, 1968). The degradation rate of this carbon would equal that for amino acids protected by the soil constituents rather than that of cellulose. Although no known mathematical models utilizing this principle have been constructed for soil organic matter to date, this should be possible using systems similar to that of the decay rates of secondary radioactive isotopes (Friedlander et al., 1964).

If continued long enough to give an estimate of the breakdown of the more resistant fractions, the data from tracer experiments are especially useful for describing the turnover of soil constituents. Soil organic matter decomposition curves such as shown in Figure 4 can be resolved into a number of exponential functions which yield straight lines when plotted on semilogarithmic paper. Immature oat residues were added to a Black Chernozem and incubated for 666 days. By measuring the radioactivity of the evolved CO_2 and of the residue, it was possible to determine the percent plant tissue remaining in the soil at a specific time.

The turnover rate of the fractions represented by the straight lines on the graph can be expressed by their half-life. This was found to be 802 days in the laboratory for the resistant fraction. The curve was readily separated into two more components indicat-

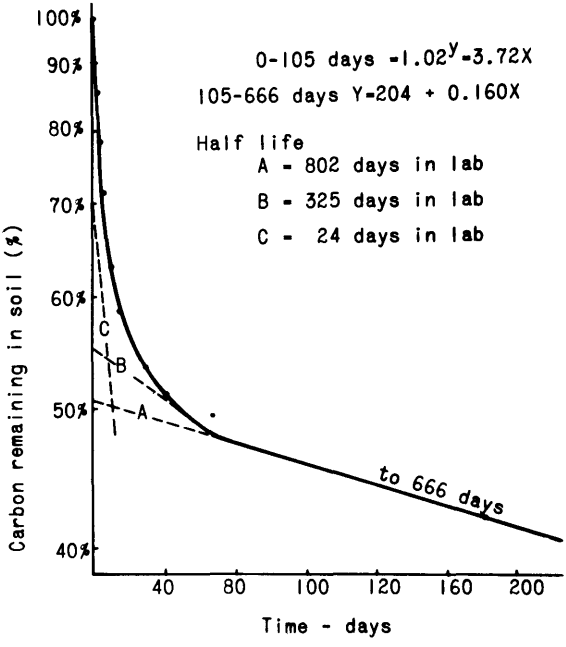

Fig. 4. Decomposition of oat residue in Chernozem.

ing a moderately resistant fraction with a half-life of 325 days and a nonresistant fraction with a half-life of 25 days. Extrapolation of these laboratory results to field conditions, by using Q_{10} factors and accounting for differences in moisture content, indicates that the resistant material would have a half-life of approximately 10 years in the field under the semiarid, cool conditions of Saskatchewan. This is twice as much as that found for similar material under field conditions in Southern England (Jenkinson, 1965) where the half-life of the resistant, added material approximated four years while the average half-life for the unlabeled carbon was 25 years.

The above discussion has indicated that the majority of soil constituents reside in the soil for extensive periods of time. The common tracer techniques run into difficulty in that, although both nitrogen and carbon tracers can be used over long periods, it is difficult to establish adequate equilibrium conditions in short-term experiments. The normally used tracer techniques, therefore, will continue to prove most useful in studying those fractions of soil organic matter which have high turnover rates. These fractions are

important from an energy flow and nutrient cycling viewpoint but they do not contain the majority of the organic components. Other techniques must be used for studying the turnover rate of the resistant fractions to interpret their role in geochemistry and pedogenesis.

THE APPLICATION OF CARBON DATING TO SOIL ORGANIC MATTER INVESTIGATIONS

Bombardment of the atmosphere by cosmic radiation produces high energy secondary neutrons which react with nitrogen to produce ^{14}C. The ^{14}C is oxidized to $^{14}CO_2$. This is mixed by winds with inert $^{12}CO_2$ and $^{13}CO_2$. Of the total CO_2 photosynthesized, 1 part in 10^{10} is $^{14}CO_2$, and 1.1 part in 100 is $^{13}CO_2$. In addition to the ^{14}C transferred to the biomass, a significant amount remains in the atmosphere and a large amount is transferred to the hydrosphere (Libby, 1955).

Libby (1955) predicted, and later proved that the distribution of radiocarbon through the dynamic reservoir was fairly uniform and that the cosmic ray intensity has been essentially constant for at least 20,000 to 30,000 years. By measuring the radiocarbon activity of a sample and that of a modern reference sample it is possible to estimate the sample's age from the following equation:

$$A = A_o \cdot e^{\frac{-0.693t}{5568}}$$

Where A_t = radiocarbon activity of the sample at time t (in years) after death
A_o = radiocarbon activity of the sample at death
e = base of natural log
5568 = half-life of ^{14}C in years

There are a number of factors which affect carbon dating. Isotopic discrimination (Craig, 1954) and the Suess effect (Suess, 1955), which results from the dilution of radiocarbon in the atmosphere by the nonradioactive carbon of fossil fuels, must be considered. The atmospheric testing of hydrogen bombs resulted in the production of fairly large concentrations of ^{14}C (Broecker and Olson, 1960). In addition there are some variations in the natural ^{14}C content in different parts of the world (de Vries, 1958). This

technique has been found reliable in determining the age of a wide cross-section of organic samples such as materials from archeological sites, tree rings, buried soil profiles, and peats (Libby, 1955; Scholtes and Kirkham, 1957; Barker, 1958; Blackburn, 1966).

The humus of the soil profile differs from other carbonaceous residues in that incorporation of the ^{14}C from the atmosphere via plant residues continues at all times. This mixture of materials, deposited over thousands of years, is continuously undergoing degradation to at least some extent. In carbon dating surface soils, one does not obtain a specific age of the carbon present but a 'mean residence time' (MRT) (Paul et al., 1964).

Simonson (1959) has quoted ages ranging from 100 to 400 years for the organic matter of some cultivated soils in the north central United States. Tamm and Holmon (1967) have recently summarized the data obtained from carbon dating a number of European forest soils. It was found that the organic matter in the Podzolic B horizons generally had a MRT ranging from 330 $\pm$ 65 to 1260 $\pm$ 60 years with the higher values being found in the more northern regions. The surface litter and A_1 horizons, although having a low MRT (approximately 100 years), were old enough to confirm the opinion that the A horizon humus is, to a large extent, the product of the last forest generation. A number of other carbon dates have shown the applicability of this technique to pedological and ecological analyses (Perrin et al., 1964; Vasari, 1965; Scharpenseel et al., 1968).

Discrimination against ^{13}C (Craig, 1954, 1957) and against ^{14}C (Sorokin, 1960) has been shown in a number of photosynthetic, chemical, and microbiological systems. The bonds involving ^{12}C (the more energetic ones) react preferentially to those containing the heavier ^{13}C isotope. In the decarboxylation of n-malonic acid, those molecules containing only ^{12}C reacted 3.3 percent faster than molecules containing one ^{13}C, with the $^{12}C/^{12}C$ bond, being ruptured 2 percent more frequently than the $^{12}C/^{13}C$ bond (Lindsay et al., 1951). ^{13}C depletions ranging from -5.5 to-12.8°/$_{00}$ have been observed in the CO_2 released during growth by *Desulfovibrio* on lactate (Kaplan and Rittenberg, 1964), and both $^{13}C/^{12}C$ and $^{34}S/^{32}S$ ratios have been altered by cultures of *Salmonella heidelberg* during anaerobic SO_3 reduction in Trypticase broth (Krouse and Sasaki, 1968).

Small variations in the relative abundance of nitrogen isotopes also have been observed in nature (Hoering, 1956; Parwel et al.,

1957; Bremner, 1965b), the observed range of variation of 1.5 percent is half that observed for the stable carbon isotope ^{13}C. Although discrimination is usually not taken into account in laboratory investigations, this factor must be considered in systems where the substrate is subjected to a fair degree of cycling which could cause discrimination.

The $\delta^{13}C$ value, determined mass spectrometrically, has been found to be fairly constant for a range of soils and humic fractions analysed (Table 8). Relative to the Solonhofen standard, the $\delta^{13}C$ values range from -21.5 to -24°/$_{00}$. These values are similar to those reported by Broecker and Olson (1960). This discrimination against ^{13}C (with that against ^{14}C being twice that of ^{13}C) (Craig, 1954; Broecker and Olson, 1959) may have occurred either during the physiological processes of the plant or the transformation of plant materials to humus, or during both processes. The $\delta^{13}C$ of wheat grown on the soils analysed showed little difference from that of the soil. This suggests that discrimination against the heavier carbon isotopes by soil organisms is small relative to that occurring during plant growth.

The explosion of hydrogen bombs in the atmosphere during the 1950's and early 1960's has increased the ^{14}C content to the extent that it has more than overcome the "Suess effect" caused by the combustion of nonradioactive coal and oil products (Suess, 1955; Broecker and Olson, 1960). The MRT of the Melfort soil sampled in three years, when the radioactivity of bomb carbon was rapidly rising, and of the Regina soil sampled in 1952 and again in 1964 shows no effect from the bomb carbon. This can be attributed to one or all three of the following factors: 1. the high carbon content of the soils; 2. the rapid turnover rate of fresh organic residues in soils; and 3. the relatively low MRT of the soil humus (Campbell et al., 1967a, b). These points can be demonstrated by calculating the apparent decrease in mean residence time which would accompany the incorporation of the residue of an average wheat crop into the Melfort soil. Figure 5 shows that the closer the MRT of the soils and the added residue, the less significant will be the alteration in radiocarbon activity by the addition of a fairly large concentration of contemporary carbon. Assuming that 1,500 pounds per acre of carbon are added to the soil during a normal growing season, a residue of 450 pounds per acre would be left after the first year if 70 percent had decomposed (Lueken et al., 1962). This corresponds to 0.4 percent of the total carbon in the Melfort soil analysed and the

TABLE 8

The Use of $\delta^{13}C$ in the Normalization of Mean Residence Times of Soils and Humic Fractions (Campbell et al., 1967b)

Sample	$\delta^{14}C$ (‰)	Apparent mean residence time (year)	$\delta^{13}C$ relative to N.B.S. Solenhofen stand. (‰)	Normalized mean residence time (years)
Melfort soil 0-6″ depth	−115.5 ± 6.0	990 ± 60	−23.1	870 ± 50
Melfort soil 6-10″ depth	−130.8 ± 7.7	1130 ± 70	−26.3	960 ± 65
Waitville soil	−43.8 ± 7.6	360 ± 65	−22.6	250 ± 60
Oxbow soil	−120.5 ± 7.5	1030 ± 70	−21.5	940 ± 60
Fractions of Melfort soil				
Humin II	−146.1 ± 6.3	1270 ± 60	−23.9	1140 ± 50
Humic acids II	−154.8 ± 7.3	1350 ± 65	−23.6	1235 ± 60
Fulvic acids II + acid extract	−70.0 ± 11.5	585 ± 95	−23.0	470 ± 90
Nonhydrolysable Humin II	−155.0 ± 7.1	1350 ± 60	−23.2	1230 ± 60
Nonhydrolysable Humic acid II	−171.2 ± 7.0	1510 ± 65	−22.6	1400 ± 60
Humic acid II-hydrolysate	−13.25 ± 6.3	110 ± 60	−21.1	25 ± 50
1952 wheat sample	–	–	−20.0	–

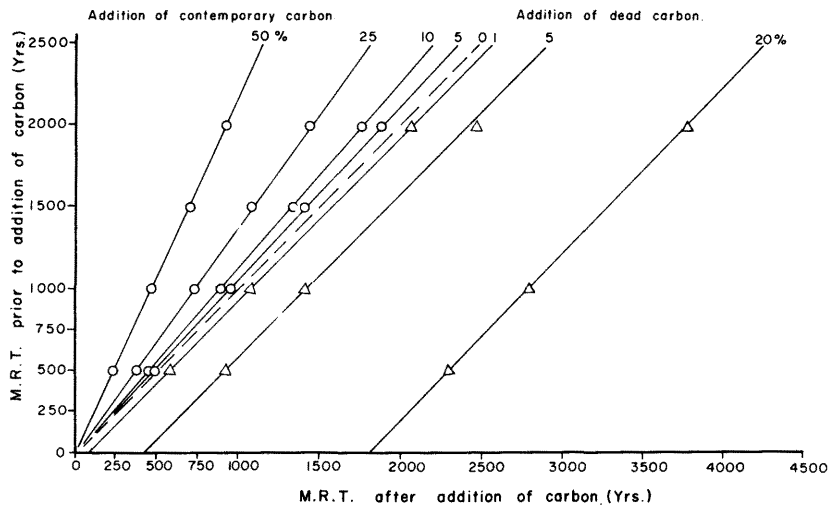

Fig. 5. Effect of added carbon on the "true" m.r.t. of a sample. (From Campbell et al., 1967a. Soil Sci., 104: 81-85.)

change in mean residence time of the soil humus by the added carbon is within the error of measurement. However, the accumulative effort could be of significance in soils of lower organic matter content (Campbell et al., 1967a, b).

The possibility exists that direct assimilation of soil CO_2 by plants might be a source of error in carbon dating studies of soil. The amount of soil CO_2 absorbed by roots is only about 2 percent of the total CO_2 assimilated by the plant. Most of the soil CO_2 utilized by plants in the photosynthetic process is mixed with the atmospheric CO_2 before the plant uses it (Musgrave and Moss, 1961). In addition, the ^{14}C activity of the CO_2 evolved from the soil is only slightly lower than that of the atmospheric CO_2 (Broecker and Olson, 1960).

The information that the humus of Podzolic (Waitville) soils had a mean residence time of 250 $\pm$ 60 years, whereas the Chernozemic dated approximately 860 years and a number of Brown soils dated around 400 years, is very useful in studying the genesis of these soils. The humic material associated with the clays was the most persistent in the Podzolic which has undergone leaching of the cations from the surface horizons (Campbell et al., 1967b). If meaningful data relative to the turnover rates of the soil constituents are to be calculated, the humus must be fractionated into

TABLE 9

Estimate of Carbon and Nitrogen Released Per Annum from the Humus of the Melfort Soil

	Mean rate of decomposition*	Carbon lbs/ac.	Carbon released/year lbs/ac.	Nitrogen lbs/ac.	Nitrogen released/year lbs/ac.
Humic acids-hydrolysate	0.04	7840	308	1500	60
Acid extract	0.003	15680	47	1930	6
Humin hydrolysate + fulvic acids III	0.002	24640	52	3600	7
Nonhydrolysable humic acids	0.0007	36960	26	1410	1
Nonhydrolysable humin	0.0008	26880	43	1160	1
Total		112000	476	9600	75

* $r = MRT^{-1}$

meaningful components. The mean residence time of the Chernozemic, fulvic, humic, and humin portions of the soil were 470, 1230, and 1140 years respectively. This confirms previous postulations that although the classical fractionation of organic matter is useful from a pedologic standpoint, it does not adequately separate the fractions relative to their mean residence time. Hydrolysis separated the humic acid fraction into two portions, one of 1,400 years and another which was a high nitrogen component that was composed of modern carbon. Hydrolysis of the humin resulted in a fraction equal in mean residence time to the fulvic acids (Campbell et al., 1967a, b).

The preceding discussion on the use of mathematical models indicates that it should be possible to describe the dynamics of soil carbon and nitrogen if the turnover rates of its constituents are known (Bartholomew and Kirkham, 1960; Minderman, 1968). The data in Table 9 was calculated by assuming that the degradation of the different soil fractions follows first-order kinetics. Similar relationships hold for the decay of radioactive isotopes and the growth rate of bacteria during their logarithmic phase (Dawes, 1962). Bartholomew and Kirkham have shown that, if enough time has elapsed so that the materials are in equilibrium, the decomposition rate should be the reciprocal of the average age or mean residence time (Bartholomew and Kirkham, 1960).

The humic acid hydrolysate constituting 7 percent of the total carbon had a mean residence time of 25 years such that $r = 0.04$. Although the determination of this figure by carbon dating introduces a fair degree of variability, normal tracer techniques utilizing ^{14}C and ^{15}N have found a similar decomposition rate for the active fraction (Jansson, 1963; Jenkinson, 1968). Substituting 'r' into the equation:

$$C_t = C_o e^{-rt}$$

where C_t = the carbon present after time t, and

C_o = the original carbon in the fraction,

within the time t equalling one year, we find that the humic acid hydrolysate should release 308 pounds (Table 9) of carbon per year. The fractions having an intermediate mean residence time release small but significant concentrations of carbon. The humic

acid and humin residues left after 6 N acid hydrolysis make up 57 percent of the total carbon but release very little carbon on an annual basis.

The carbon and nitrogen components of the biologically active soil fractions are very closely related (Jenkinson, 1968) and it has been shown that the release of nitrogen is directly proportional to that of carbon if the carbon:nitrogen ratio of the material is low (Harmsen, 1964; Bartholomew, 1965). From the knowledge of the carbon and nitrogen content, it is possible to calculate the nitrogen released on an annual basis. The humic acid hydrolysate, although constituting 7 percent of the carbon, comprised 15.6 percent of the nitrogen. With a decomposition rate of 0.04, this fraction would release 60 pounds per acre per year annually. The only other fractions releasing significant concentrations of nitrogen are the acid extract and the hydrolysate of the humin plus the fulvic acids.

The nitrogen residue not solubilized by acid hydrolysis comprises 25 percent of the total. Since this material is turned over once every 1,300 years, it releases very little nitrogen. The carbon, nitrogen, and other nutrients in this fraction contribute to its exchange and sorption characteristics. They are, however, biologically inactive or stable with reference to energy transformations and nutrient cycling.

CONCLUSIONS

The application of instrumental and microbiological techniques has elucidated some of the structures involved in organic matter. Although there is still a disconcerting amount of substance in the column called "unidentified material" that always accompanies soil organic matter research, the amount and composition of the majority of carbohydrates, organic sulfur, and organic phosphorus products in soil is now generally known. The presence of large concentrations of free radicals in soil supports the theories concerning the role of oxidized degradation products of lignin in soil humus formation and helps explain the soil humic characteristics.

The heteropolycondensate of 3,000 to $>100,000$ molecular weight which comprises the soil humic constituents is probably formed by condensation of aromatic degradation products and amino compounds. The recent observation of larger molecular

weight amino constituents existing in the soil, with some of them at least being attached to the aromatic constituents by hydrogen bonding which can be broken by phenol and PVP, is in accordance with the information obtained by Ladd and Butler using model systems. This indicates that humic-like materials made by combining oxidized aromatics and larger molecular weight nitrogeneous constituents more closely resemble the humic acids than when amino acids are incorporated exclusively as single units. Direct evidence for the occurrence of peptide bonds in soil humic acid has recently been shown by the action of the proteolytic enzyme, pronase, on both model and natural humic materials.

The formation of soil humic material is dependent on the phytochemicals entering the soil, the microbial population, and soil characteristics such as pH, extent of aeration, and content of stabilizing clays and cations. Humic materials from different parts of the world, although being similar, have characteristics dependent on the environment in which they were formed. Dubach et al. (1964) have stated that soil organic matter research could progress rapidly if laboratories in different parts of the world used exactly the same material. The alternative is to take into consideration the strong influence of environmental and soil factors in the formation of humic materials. This requires close collaboration with pedologists in collecting soil samples so that typical materials are utilized.

The dynamics of the soil organic matter are being investigated using long-term field experiments, and by the use of tracers. Normal tracer techniques will continue to be the backbone of this research for they are available to a great number of laboratories and characterize the active portion of soil humus. The use of carbon dating makes it possible to characterize the indigenous soil fraction which cannot be studied by normal techniques.

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