

Lectures on
Soil Organic Matter

by

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Foreword

To my friends and the friends of the subject.

In this manuscript are some unpublished results and therefore only for friends and not to use for publication.

I would enjoy having any comments on this material.

W. Flaig

Acknowledgments

This manuscript came about as a result of the kind invitation of Prof. Dr. W. H. Pierre, Head, Department of Agronomy, to give lectures about soil biochemistry. I am very thankful for this opportunity.

I would like to express my best thanks also to my colleague, Prof. Dr. Lloyd Frederick, who stood by me helpfully at all times during the writing of these lectures in the English language and I appreciate his suggestions during our many discussions.

Without the help of his co-workers, Messrs. McIntosh Sims, Horton, Brown, and of the secretaries, Mrs. McLaughlin, Misses Sansgaard and Zart, it would not have been possible to mimeograph the lectures. Also to these, many thanks.

June, 1959

W. Flaig

SOIL ORGANIC MATTER

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The decomposition of known compounds of plant residues and isolation of compounds with definite chemical constitution

In the next part of the lecture we will speak about the decomposition of known compounds of plant residues and the isolation of compounds with a definite chemical constitution.

The organic soil substances come mainly from dead organisms. The most important amount of residues originate from plants, a lesser one from animals and microorganisms

Amount of residues year/ha	Plants	Animals in the soil	Microorganisms
	4000-5000 kg	70-400 kg	2000 kg
	Proteins aliphatic u. aromatic amino acids	Carbohydrates Sugar	Fats Aliphatic acids org. phosphoric compounds
	Lignin Phenolic comp. Pectins Waxes	Amino sugar Chitin	Special metabolism products dyestuffs (pigments) Comp. of quinonic structure Antibiotics

Organic compounds of plants, soil animals and microorganisms important for decomposition of residues.

A number of chemical compounds is similar in plants, in soil animals and microorganisms. They use them to compose their body substances. All organisms consist of proteins, of aliphatic and aromatic amino acids, of carbohydrates, sugars, fatty compounds, aliphatic carbonic acids and different organic phosphorus compounds. During our considerations it is necessary to speak about substances which are more or less special for certain organisms. One of the most important substances in plants for the humification is lignin and phenolic compounds.

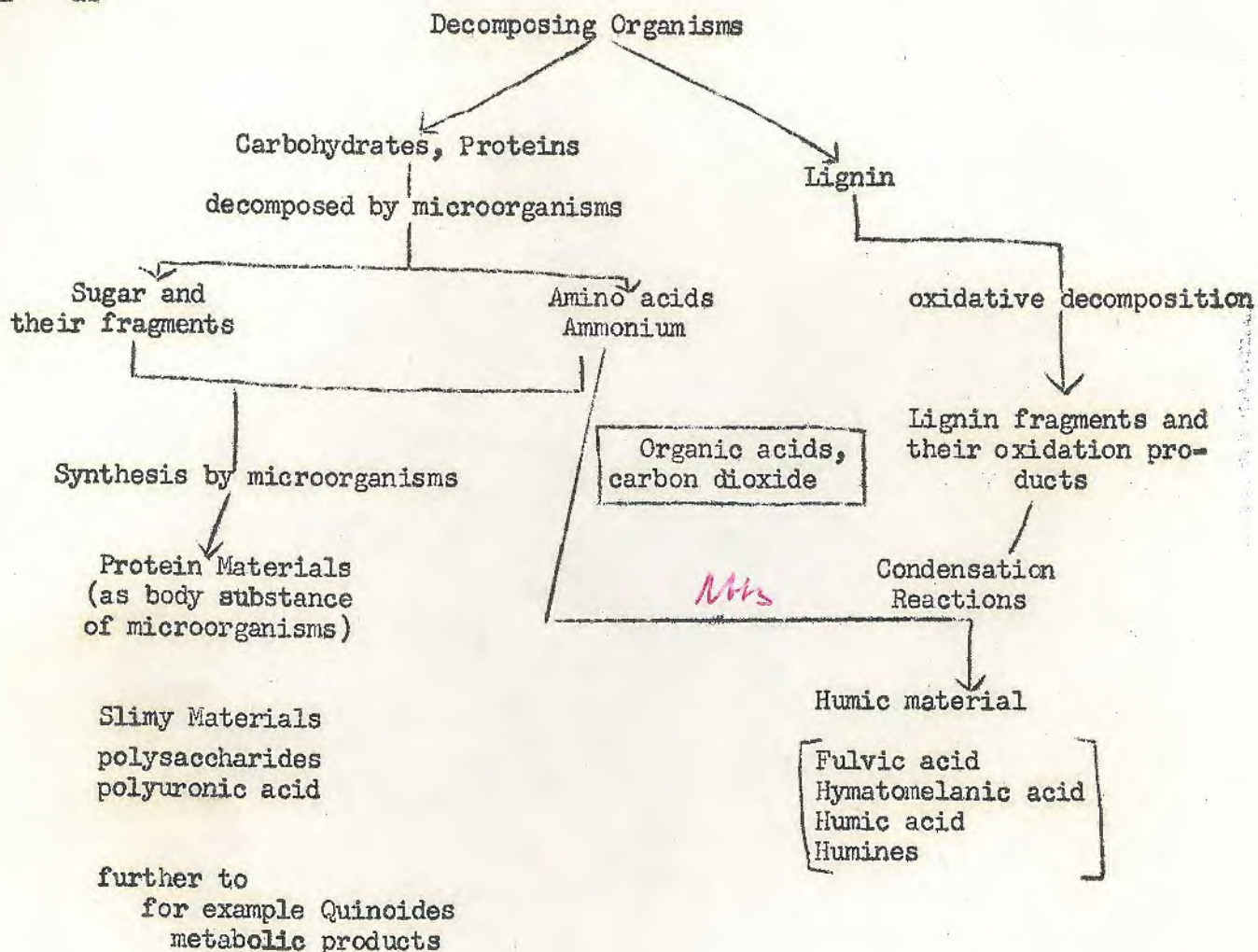
In a lot of soil animals is an important constituent chitin. Bremner (Reprint 102, 1) suggest that 5 up to 10% of the total nitrogen in soils belongs to amino sugar complexes. Others have had the same results.

The number of the special metabolism products in microorganisms is very large. Of only a few we will speak, which are interesting for the processes of humification. Some of the microorganisms produce pigments which have a quinonic structure. (102,2)

One of the newest lines of research is to study the effect of antibiotics on the plant growth. The high molecular substances decompose more or less in the soil during the transformation processes for humification. According to investigations made for very different purposes one can conclude that the building blocks of those high molecular substances, which are important for the humification, decompose nearly all by the aid of microorganisms. The rate of decomposition is different. Aliphatic amino acids and nearly all aliphatic acids, the monomer sugars and their derivatives decompose more rapidly than the aromatic amino acids and the heterocyclics such as purine and pyrimidine, phenolic compounds and lignin are relatively resistant to decomposition. For all these transformations the enzymes which are in the soils play an important role (102,3).

Carbohydrates and proteins make up the largest part of the dead organisms. The content of lignin in the plants varies and is for grass nearly 10%, for straw 20%, and in different kinds of wood, an average of 30 up to 35% of their dry weight.

S 1 12



In the following scheme, the most important decomposition reactions and transformations of the organic substances in soil are shown. Carbohydrates and proteins are split off by the microorganisms relatively quick. They are used for their metabolism.

Lignin is more resistant against the attack of microorganisms. (102,4). If cellulose decomposes more and more, lignin can be oxidized (102,5). By these reactions fragments of lignin and their oxidation products are formed. The carbohydrates decompose also. Sugars and their fragments are formed. In the same manner, from the proteins come amino acids and ammonia.

The humic substances are formed by the condensation reactions of lignin fragments and their oxidation products together with fragments of carbohydrates and proteins, such as sugars and amino acids and ammonia. The decomposition of organic substances in soil is accompanied by the formation of different organic acids and carbon dioxide (102,6). The microorganisms do not only decompose the different organic compounds in the soil but they also form new proteins as their body substances as well as slimy substances such as polysaccharides and polyuronides. Some special

metabolism products such as compounds of quinonic structure are also formed.

By this scheme, the important fact results that the humic substances do not come in the soil with the dead organisms but are synthesized in the soil during the decomposition of organic substances. First of all in this scheme is shown the way of forming humic acids out of lignin. Other possibilities of their formation will be discussed later on. But in principle there is no difference.

At first we will study those kinds of substances with known chemical constitution which come into the soil. Furthermore, we will see what kind of transformations are going on and what kind of definite substances have been found.

The important work in this field has been done under the leadership of Schreiner and Shorey. They give a lot of methods for the isolation and identification of the humus constituents and knowledge of the free substances, which have become definitely identified. An outline of the general procedure employed in this investigation on the humus of soils and peats to determine different substances is shown in the next table. These kind of investigations gain more and more interest as the years go by.

§. 103

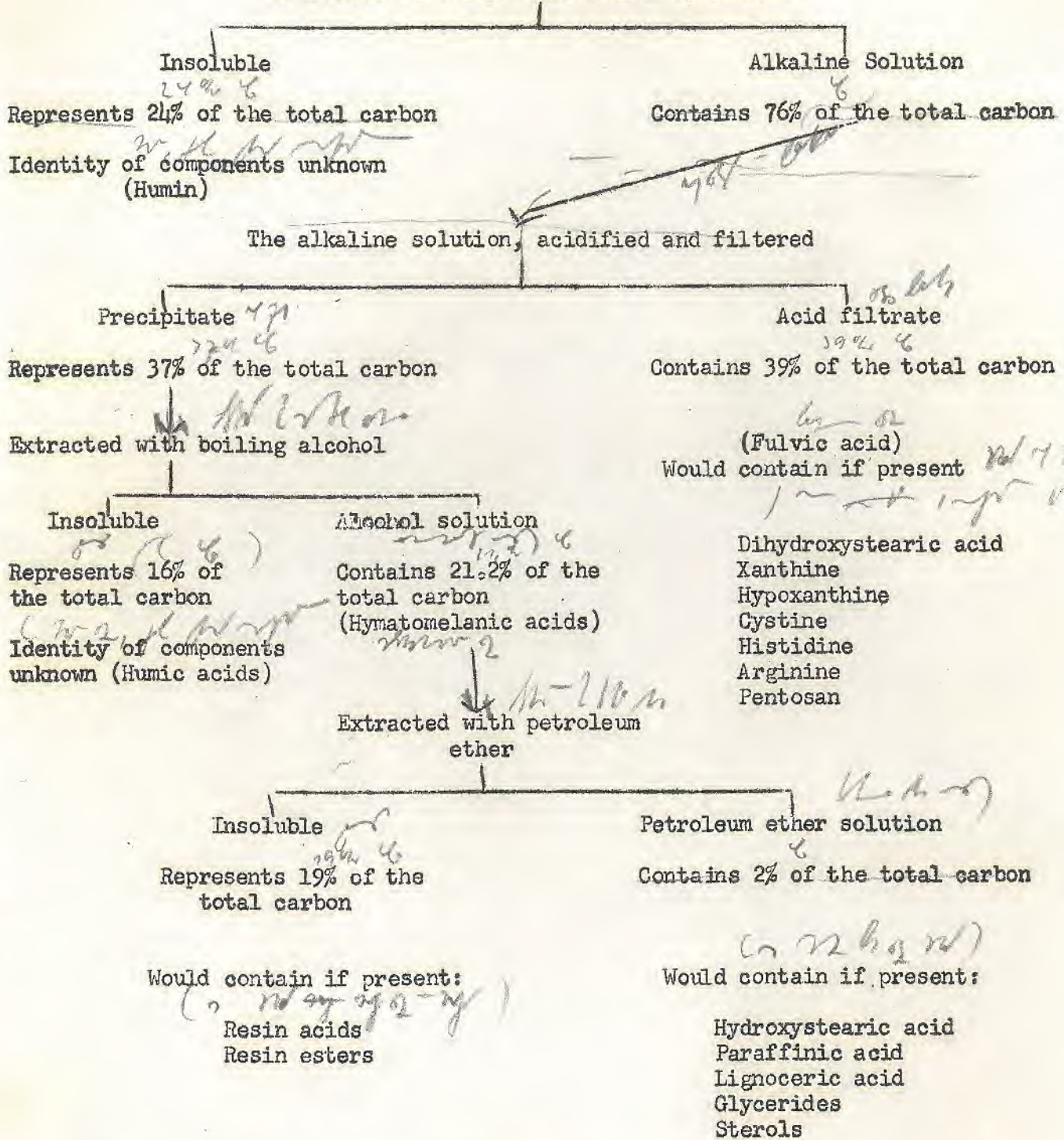
The raw material had been a mineral soil with 0.95% organic carbon (equal to 100 part total C for the further calculations). By extraction with sodium hydroxide, the organic components have been divided. (2% NaOH)

- I. Insoluble organic parts contain 24 parts of total C. Composition unknown. So-called humin.
- II. Organic part soluble in alkali contains 76 parts of total C. It is divided by acidification and filtration.
 - a. Acid filtrate contains 39 parts of total C. The largest amounts are not identifiable, but there are some definite substances. According to Sven Oden, this fraction is called fulvic acid.
 - b. Precipitate contains 37% of total C. Can be divided by boiling with alcohol.
- I. Part soluble in alcohol contains 21 parts of total C. So-called hymatome-lanic acid. Can be extracted with petroleum ether.
 - (A) Part insoluble in petroleum ether contains 19% of total C. From this fraction some components of wax can be isolated.
 - (B) Part soluble in petroleum ether contains 2% of total C. From this there could be isolated numerous substances.
- II. Part insoluble in alcohol contains 16% of total C and is of unknown composition. So-called humic acids.

Some of the substances are known to be present in organisms, some are not. In some cases it may be that the one or other substance is formed by the method of isolation.

Distribution of organic compounds in soil humus (Schreiner and Shorey)

Soil containing 0.955 percent organic carbon was extracted with 2 percent sodium hydroxide



All the substances were found in very low amounts, in some cases only a few milligrams per 100 grams soil. Therefore one believes that these substances are, in most cases, intermediates of the humification. If also the content is small in percent, the amount in the surface soil is nearly of the magnitude of fertilizing with mineral nutrients. An effect on plant growth or on the development of the organisms in the soil may be therefore out of the question, insofar as these substances have a biological effect.

The isolated and identified substances are:

(A summary is given by J. H. Waksman: Humus, Origin, Chemical Composition and importance in nature, The Williams and Wilkins Company, Baltimore, (1936), p. 129-152)

Alcohols

Mannitol (Bull. 88) $CH_2OH (CHOH)_4 CH_2OH$

Sugars

Rhamnose (Methylpentose) (Bull. 88) $CH_3 (CHOH)_4 \cdot CHO$

This sugar has been found as a glucoside and was present in comparitively large concentration in the colored material precipitated by lead acetates from the acid filtrate of alkali extract of soil.

Acids

Oxalic acid (Bull. 88) $\begin{matrix} COOH \\ | \\ COOH \end{matrix}$

This has been found in the acidified filtrates of the alkaline extract of soil after treating the soil with dilute hydrochloric acid. Shorey suggested that this acid is present in the soil in the form of calcium oxalate, frequently in higher concentrations, but it appears occasionally.

Succinic acid (Bull. 88) $\begin{matrix} COOH \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{matrix}$

The fraction obtained after neutralization of the alkali extract of the soil with sulfuric acid and removal of the precipitate contained this complex.

Saccharic acid (Bull. 88) $HOOC-(CHOH)_4 \cdot COOH$

It is widely distributed in the soil. This compound is not known to occur in plant or animal tissue. One believes that it is formed through the action of microorganisms in the soil.

Acrylic acid (Bull. 88) $CH_2 = CHCOOH$

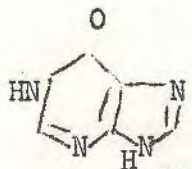
It is suggested that this compound is formed by microorganisms because it doesn't occur in plant and animal tissue.

Crotonic acid (Walters, E. H. u. L. E. Wise: \sphericalangle Crotonic acid a soil constituent. J. Agricult. Res. 6 (1916)

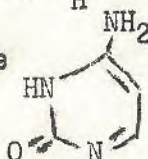


94 mg could be isolated from 50 pounds of a sandy loam soil from Texas. In his book, Waksman suggested that this compound could be formed during the decomposition of cellulose by bacteria in the transformation of hydroxy acids of the aliphatic series or by hydrolysis of allylcyanide, which is present in the essential oils of certain plants.

Hypoxanthine, 6-oxy-purine

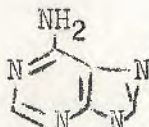


Cytosine, or 2-oxy-6-amino-pyrimidine



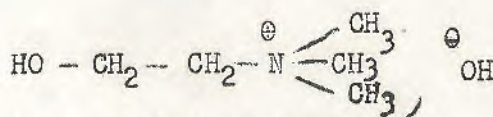
The last three derivatives have been found by Schreiner, O. u. E. C. Shorey: Pyrimidine derivatives and purine bases in soils. J. of Biol. Chem. 8 (1910)

Adenine, 6-amino-purine (Bull. 88)



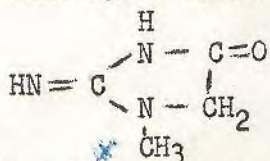
The last four compounds are fragments of nucleic acids. They could have been introduced into the soil by plant residues or by microorganisms.

Choline (Bull. 88)



This compound had only been found in low concentration.

Creatinine (983) Schreiner, O., Shorey, E. C., Sullivan, M. X. and Skinner, J. J.: A beneficial organic constituent of soils: Creatinine. U. S. Dept. Agr. Bur. Soils, Bull. 83. 1911. (1008) Shorey, E. C.: The isolation of creatinine from soils. Jour. Amer. Chem. Soc. 34:99-107. 1912. (1018) Shorey, E. C.; Sullivan, M. X. and Skinner, J. J.: A beneficial organic constituent. Creatinine. U. S. Dept. Agr. Bur. Soils. Bull. 83. 1911.

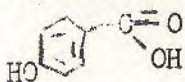


Derivatives of benzene (Shorey, E. C.: The presence of some benzene derivatives in soils. J. agricult. Res. 5. (1916)).

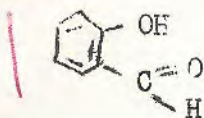
Benzoic acid 

2 gm of this acid were obtained from 25 kg by extracting the acid filtrate of the acidified alkali extract with ether.

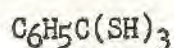
p-hydroxy-benzoic acid (Walters, E. H.: The isolation of p-hydroxy-benzoic acid from soils. J. Amer. Chem. Soc. 39 (1917)).



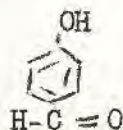
Salicylic aldehyde (o-Oxybenzaldehyde) Bull. 88)



Tri-thio-benzaldehyde (Bull. 88)

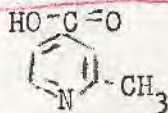


Vanillin



25 kgs of soil yielded only a few milligrams of this compound. It can also be isolated by extracting the soil directly with alcohol. It seems to be an oxidation product of lignin.

Heterocyclic compounds



α -picoline- β -carboxylic acid; (Bull 53. Schreiner, O. and E. C. Shorey: The isolation of picoline carboxylic acid from soils and its relation to soil fertility. J. Amer. Chem. Soc. 30, pp. 129501307. (1908).

Furthermore, there has been isolated phosphorus compounds such as nucleic acids and inositol phosphates. We will speak later on in a more detailed manner about these kinds of components.

Together with the plant residues different kinds of high molecular substances get into the soil. The isolation of high molecular substances is much more difficult than that of low molecular ones. Therefore, it is only possible to study the decomposition of pure high molecular substances added to the soils, and to determine the rate of transformation or to split up the high molecular substances into their building blocks and to isolate them. With time for each case, an example shall be given.

Out of the numerous papers about the decomposition of cellulose in the soils we will refer to more recent results. E. L. Schmidt and O. R. Ruschmeyer studied the cellulose decomposition with a soil burial test. Fabric strips are buried in the soil and exposed to the activity of the cellulolytic microflora. The cellulose decomposing ability was measured on the one hand by the quantity and rate of evolution of carbon dioxide during the decomposition of fabric samples of cellulose, and on the other by the decrease in tensile strength of fabric strips in the course of a standard soil burial test procedure. Untreated cotton duck was used as cellulosic material.

The properties of the soil have extensively been determined, and correlated with the decomposition of cellulose. This can be compared with the given tables.

(Tables 8, a,b,c.)

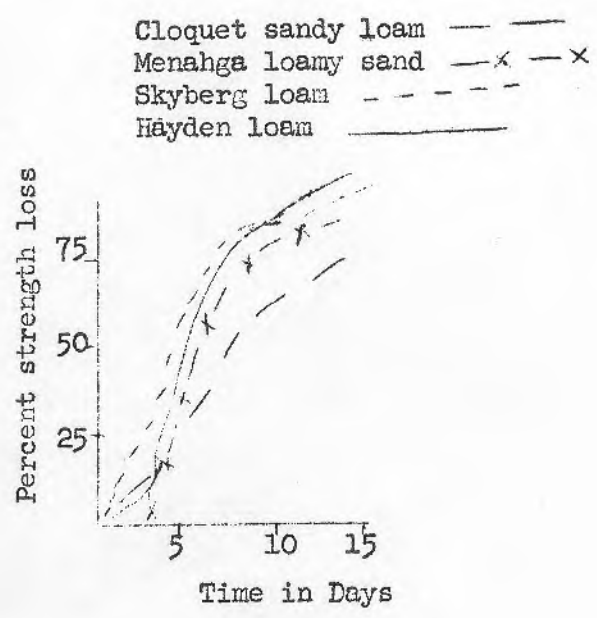
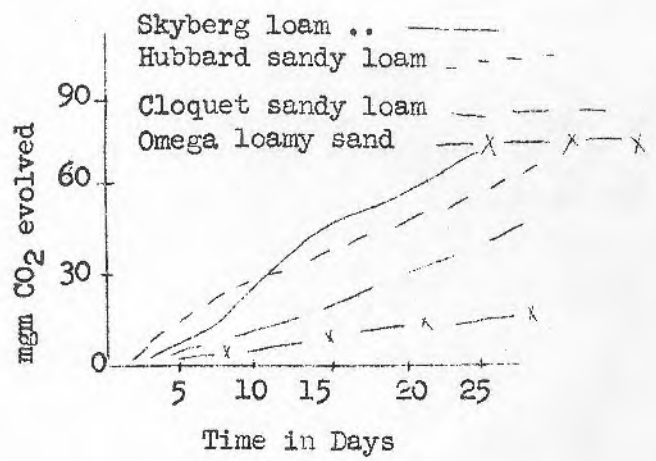
Without details I report the results. The correlation coefficients (r-value) between soil factors and cellulose decomposition, either the cellulolytic activity measured by CO_2 evolution or measured by the soil burial test, are summarized in the following table (Page 9).

PROPERTIES OF THE SOILS MENTIONED

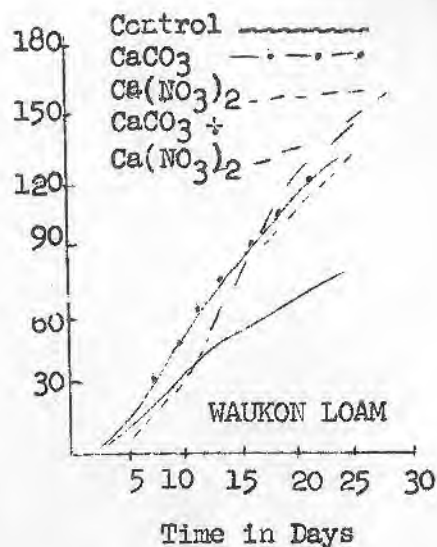
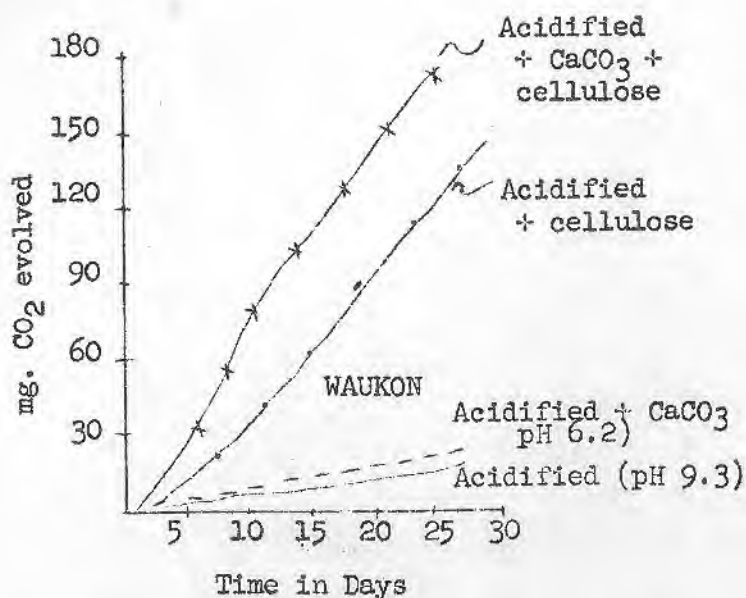
<u>Physical and Chemical</u>	<u>Cloquet Sandy Loam</u>	<u>Skyberg Loam</u>
<u>Texture Analysis</u>		
Sand (%)	68	30
Silt (%)	24	48
Clay (%)	8	22
Moisture Equivalent (%)	21	25
pH	4.0	5.3
Organic Carbon (%)	1.81	1.65
Total Nitrogen (%)	0.110	0.194
C:N Ratio	16.5	8.5
Nitrate Nitrogen	0.7	22.3
Nitrifying Capacity (NO ₃ -N) (ppm)	1.0	19.0
Available Phosphorus (P) (ppm)	14.5	10.0
<u>Microflora</u>		
Cellulose Decomposers (per gram)	1,160	139,000
<u>Cellulolytic Activity: Soil Burial Tests</u>		
Percent Loss in Strength after days		
3	4.0	14.3
5	30.0	52.1
8	56.0	82.4
14	75.0	94.9
Area under Curve of % Loss in Strength in Soil Burial for 14 Days (sq. in.)		
	4.5	6.6
CO ₂ -C Evolution Tests - Area Under Curve for 28 Days (sq. in.)		
	7.4	13.1

Schmidt, E. L., and O. R. Ruschmeyer. Cellulose Decomposition in Soil Burial Beds.
I. Soil Properties in Relation to Cellulose Degradation. Applied Microbiol.
Vol. 6, No. 2. March, 1958.

Ruschmeyer, O. R., and E. L. Schmidt. Cellulose Decomposition in Soil Burial Beds.
II. Cellulolytic Activity as Influenced by Alteration of Soil Properties.
Applied Microbiol. Vol. 6, No. 2. March, 1958.

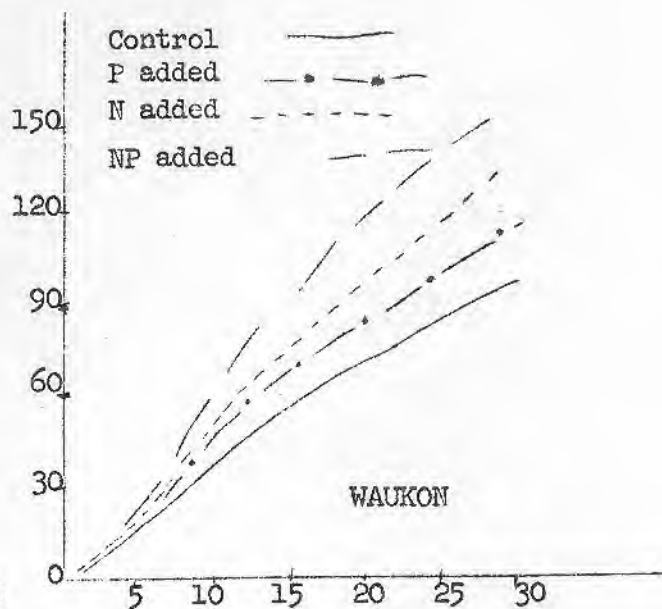
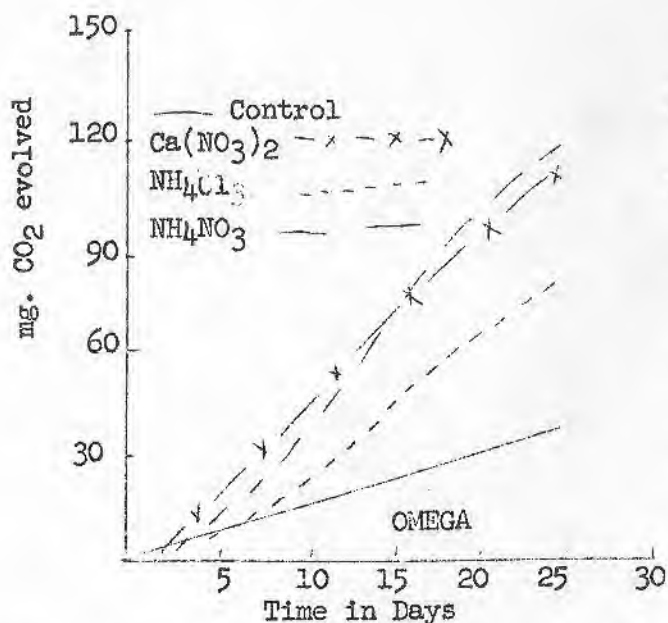


V. Curves representative of soil of different cellulolytic action.



I. Effects of pH change on CO₂ evolved from cotton duck buried in acidified portions of soil normally nearly neutral in reaction. Curves are presented for untreated and limed Waukon silt loam after acidification.

II. Effect of pH and nitrate on the CO₂ evolved 1 g. strips of cotton duck buried in control and untreated samples of acid soil.



III. Curves of CO₂ evolved for Omega loamy sand as affected by additions of inorganic nitrogen. 1% cellulose present in all soil samples.

IV. CO₂ evolved from Waukon silt loam as affected by treatments of potassium phosphate and calcium nitrate. 1% cellulose added to the samples.

Correlation coefficients (r-value) between soil factors and cellulose decomposition

Soil Factor	Cellulolytic activity measured by CO ₂ -C evolution*)	Cellulolytic activity measured by soil burial tests +)
Sand content	-0.47	-0.41
Silt content	+0.47	+0.41
Clay content	+0.41	+0.50
Moisture equivalent	+0.57*	+0.40
pH	+0.76*	+0.67*
Organic carbon content	+0.50	+0.31
Total nitrogen content	+0.63*	+0.44
C:N ratio	-0.51**	-0.50
Nitrate nitrogen	+0.57*	+0.59*
Nitrifying capacity	+0.69*	+0.58*
Available phosphorus	-0.38	-0.13

+) r-value between CO₂-C evolution and soil burial - 0.73

* significant at the 1% level

** significant at the 2% level

From this table it can be seen that soil reaction and the nitrogen relationship of soils are most important factors which influence the activity of the cellulolytic microflora.

These results agree with the investigations of Waksman (1932; Waksman and Hutchings, 1935; and White, et al., 1934 and 1949).

No work has been done until now from which can be maintained that free sugars have been found in soils. Only methylglyoxal (CH₃COCHO) a fragment of sugar had been found by Enders C. Enders and K. Theiss: *Brénnstoff-Chemie B. C.* 19, 360-365, 402-407, 439-449 (1938). Enders, C. *Über den Chemismus der Huminsäurebildung unter physiologischen Bedingungen.* *Biochem. Z.* 313, 352 (1943). - *Wie entsteht der Humus in der Natur,* *Die Chemie* Nr. 41/42 (1943). But this was isolated in such small amounts that one can have some doubt of the identification.

(Table 9a)

It was possible to isolate derivatives of sugar. (Bremner (1949, 1950, PDB 69, page 38 - Bremner, J. M. and Shaw, K.: *J. Agric. Sci.* 44, 152 (1954). Bremner hydrolyzed soils with 6 N HCl at 170° for 6 hours (compare table). The six soils had a nitrogen content ranging from 0.17-2.82%. The ammonia nitrogen content present in the hydrolysates was estimated by micro-diffusion with MgO at room temperature. The amino sugars split off nitrogen after boiling with alkali buffer solution. The distilled ammonia was determined. By subtracting the first determined ammonia content from the total the amount of amino sugars could be estimated. The soils vary in their content of amino sugar nitrogen. The amino sugars could also be identified by paper chromatography in the hydrolysates of the soils. Bremner's results indicate that 5-10% of the total nitrogen in the soil is in the form of amino sugars. Free glucosamine is probably not in the soil. Chitin added to soils is split by hydrolysis to glucosamine nearly quantitatively.

The microbial decomposition of amino sugars in soils has also been studied with chitin and glucosamine. These and other substances have been incubated in the soils under conditions found to produce rapid nitrification of ammonium sulfates. The results showed that glucosamine and chitin are decomposed by soil microorganisms. Therefore it is plausible that the content of amino sugar nitrogen comes from living or not yet decomposed animals, mostly insects, or microorganisms. Schmidt (1936) determined the chitin content of different microorganisms (from 0.5 up to 5.6%. (Schmidt, M.: *Makrochemische Untersuchungen über das Vorkommen von Chitin bei Mikroorganismen.* *Arch. Mikrobiol.* 7, 241-260. (1936).

Estimation of Amino Sugar-N in Soils by Alkaline Decomposition and Colorimetric Methods.

Soil No.	pH	Percentage in Oven-Dried Soil		Amino Sugar-N in Soil Hydrolysates*)		Colorimetric Methods		Mean	Amino Sugar-N in Soils*
		N	CaCO ₃	A	B	C	D		
1	7.7	0.23	2.39	4.7	6.5	5.7	-	5.6	7.0
2	6.9	0.43	1.29	3.9	4.1	3.8	-	3.9	4.9
3	6.3	0.17	0	5.1	6.8	6.7	5.4	6.0	7.5
4	7.1	1.50	3.04	5.7	6.0	4.9	6.0	5.7	7.1
5	6.3	2.82	0	7.0	7.5	5.1	4.1	5.9	7.4
6	4.0	2.07	0	9.4	9.7	7.6	6.0	8.2	10.2

*As percentage of total soil N

- A. Method involving distillation with pH 8.8 borate buffer (Bremner & Shaw, 1954).
- B. Method involving distillation with pH 11.1 Na₃PO₄-borate buffer (Tracey, 1952).
- C. Method of Elson & Morgan (1933) modified according to Blix (1948) and Immers & Vasseur (1950).
- D. Method of Zuckerkandl & Messiner-Klevermass (1931) modified according to Morgan & Elson (1934) and Smithies (1953).

Amino sugar-N in the soils was calculated from the mean values for the amino sugar-N contents of the hydrolysates by applying a correction factor (1.25) to allow for loss of amino sugar-N during hydrolysis.

J. M. Bremner and K. Shaw: J. Agric. Sci. 44: 152 (1954); Zeitschr. Pflanzenern. Düng. u. Bodenkunde 69: 32 (1955).

DISTRIBUTION OF AMINO ACIDS IN SOIL ORGANIC MATTER

No.	Soil	pH	Percentage on a
			Moisture-Free Basis
			N
1	Fen	7.3	2.38
2	Fen	7.2	2.62
3	Fen	7.5	2.04
4	Fen	7.2	1.52
5	Clay loam	7.7	0.25
6	Clay loam	7.5	0.10
7	Peat	4.1	2.50
8	Fen	5.0	1.78
9	Chernozem	6.2	0.51
10	Clay loam	5.5	0.18

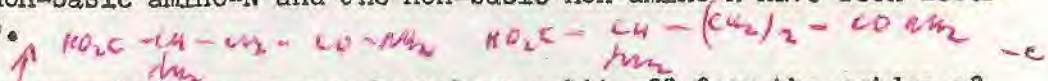
Amino Acids	S o i l n u m b e r									
	1	2	3	4	5	6	7	8	9	10
1. Phenylalanine	4	3	4	3	3	3	4	3	3	4
2. Leucine, etc*	10	10	10	10	9	9	10	10	10	10
3. Valine	9	9	9	9	9	9	9	9	9	9
4. Alanine	10	10	10	9	10	10	10	10	10	10
5. Glycine	9	9	9	8	8	9	9	9	10	9
6. Threonine	6	6	6	4	5	6	7	5	5	7
7. Serine	7	8	7	7	7	8	8	6	6	8
8. Glutamic acid	9	10	9	8	8	8	9	9	3	7
9. Aspartic acid	8	10	9	7	6	6	9	8	2	6
10. α, ϵ -Diaminopimelic acid	1	1	1	1	1	1	2	1	<1	2
11. Lysine	5	4	4	4	5	4	7	6	8	8
12. Arginine	4	4	5	4	4	4	5	5	5	6
13. β -Alanine	2	2	2	3	3	3	3	3	3	4
14. γ -Aminobutyric acid	2	1	2	2	2	3	2	3	2	3
15. α -Amino-N-Butyric acid	2	2	<1	<1	1	<1	2	2	1	<1
16. Sulphoxide of methoinine	1	1	1	1	1	-	1	1	1	1
17. Histidine	1	1	<1	1	<1	<1	3	<1	<1	<1
18. Tyrosine	2	1	1	2	2	<1	3	1	<1	<1
19. Proline	M	M	M	M	M	M	M	S	M	S
20. Hydroxyproline	M	M	W	W	W	W	W	S	W	W
<u>Amino sugar</u>										
21. Glucosamine	1	<1	2	<1	<1	-	1	<1	<1	<1

J. M. Bremner. The Amino-Acid Composition of the Protein Material in Soil. Biochem. Jour. 47: 538-542 (1950).

Now we will discuss the composition of organic bound nitrogen. The nitrogen content of the soil is relatively high and in the range 0.1-0.3%, that is, 3,000-9,000 kg N/ha to a depth of 20 cm. Of the total nitrogen content, the largest part, up to 98%, is in organic combination. At first, it must be considered in what kind of substances the nitrogen content or the content of organic combined nitrogen is fixed in the soils. About this problem a lot of work has been done.

(S 115)

I will give only 2 examples. The first is some data from Wittich, (compare table). He determined the C:N ratio of different soils. It fluctuates in large ranges between 10 and 50 to 1. The nitrogen content has been also determined. It varies also. The nitrogen has been divided according to its functional groups. After hydrolysis it could be determined that the lowest content was NH₃-N. The amino-N ranges between 25 and 60%. The humin-N is the rest of the nitrogen which cannot be determined; most of it is in heterocyclic combination. It is possible to divide the functional groups of the nitrogen in a more complete manner. I will show the results of Kojima, R. T. (Soil organic nitrogen. I. Nature of the organic nitrogen in a muck soil from Geneva, New York. Soil Sci. 64, 157-165. (1947) and Bremner, J. M. (Studies of soil organic matter. I. The chemical nature of soil organic nitrogen. J. Agricult. Sci. 39, 183-193. (1949). The α-amino-N, (Ninhydrin method) the amino and amide-N, the humin-N, the basic amino-N, the basic non-amino-N, the non-basic amino-N and the non-basic non-amino-N have been determined, (table 12).



One part of the ammonia nitrogen may have been split off from the amides of acids such as asparagine and glutamine during hydrolysis.

The α-amino N is determined with the ninhydrin method of Van Slyke, MacFadyen and Hamilton: (Determination of free amino acids by titration of the carbon dioxide formed in the reaction with ninhydrine. J. of Biol. Chem. 141, 627-680 (1941). This method is rather specific. Laatsch and Schlichting: (Zur Bestimmung der α-amino-N-haltigen Komplexe im Boden. Z. Pflanzenernahrg. Dung. u. Bodenkde 62 (107) 50-63 (1953)). We (Flaig and Th. Breyhan) found that in some cases the amount of amino-N is enlarged. The former found that MnO₂ and we quinonic and in some cases phenolic substances simulate a higher content of α-amino-N.

Composition of organic combined nitrogen in soils according to method of Hausmann

	N in % of Total N of 2 organic soils	
	Kojima (1947a) ("muck soil")	Bremner (1949a) (Fen)
α-amino-N (Ninhydrinmethode)	36.8	36.4
NH ₃ and amid-N	10.2	17.9
Humin-N	32.2	31.9
Basic Amino-N	3.8	6.1
Basic non-amino-N	17.0	5.5
Non-basic amino-N	33.0	30.3
Non-basic non-amino-N	4.3	3.1

Schlichting, E.: (Zur Kenntnis des Heidehumus. I. Fraktionierung und Untersuchung des ganzen Humuskörpers. Z. Pflanzenernahrg, Dung. U. Bodenkde 61 (106), 1-12 (1953a)), found that the amount of α-amino-N decreases with the depth of soil profiles. This is understandable because the amino-N is exclusively formed by plant residues or by the activity of microorganisms.

CONTENT AND DISTRIBUTION OF NITROGEN IN VARIOUS SOILS (According
to Nach Wittich (1952))

Boden (jeweils die A-Horizonte)	C:N Verhältnis	N-Gehalt des Humus in %	In % vom Gesamt-N		
			NH ₃ -N	Amino-N	Humin-N
<u>Parendsina auf Loess</u>	11	5.43	1.84	49.2	13.6
Mullrendsina auf Muschelkalk	12	4.79	1.46	39.9	9.2
Eutrope Braunerde auf Basalt	16	3.99	1.75	60.2	10.3
Schwach anmooriger Grundwasser- Gey (Kackreiche Wasser)	15	3.75	0.53	47.2	0.8
Braunerde mittlerer Sättigung auf Keuperlehm	16	3.53	2.55	38.0	12.7
<u>Braunerde geringer Sättigung auf Loess</u>	18	3.17	0	29.0	10.1
Podsolige Braunerde auf Flottsand ...	24	2.43	0.41	37.3	25.5
Schwach podsolierter Boden auf Flottsand	34	1.72	0	40.1	9.9
"Massig podsolierter Boden auf Flott- sand	39	1.42	3.40	29.7	13.6
"Massig podsolierter Boden auf dilu- vialem Sand	41	1.40	2.14	37.9	27.1
<u>Sphagnumtorf</u>	70	0.83	0.01	54.2	22.9
<u>Rohhumus (Kiefern 110jahrg)</u>		1.53	0.01	53.9	26.8
<u>Streu nach Abfall</u>		0.57		57.9	22.8

W. Wittich: Der heutige stand unseres Wissens vom Humus and neue Wege zur
Lösungdes des Rohhumus problems im Walde. Schriftenr. forste.
Fakultät Univ. Göttingen 4 (1952).

The humin-N in the chemistry of proteins is the part of nitrogen which cannot be hydrolyzed with boiling 6 N HCl. The chemical nature is nearly unknown. Different authors suspect that the most part is in heterocyclic combination. In comparison to the analysis of proteins the high content of this fraction in humic substances up to 33% of total N, is conspicuous.

The relatively high amount of amino-N in the total N caused many authors to try to find out the amount of amino acids in the soil. The α -amino N can originate during autolysis of microorganisms or by secretion of plant roots.

Linskens, H. F. and R. Knapp (Über die Ausscheidung von Aminosäuren in reinen und gemischten Beständen verschiedener Pflanzenarten. *Planta* 45. 106-117 (1955)) for instance found that in sterile sand cultures different amino acids are excreted by the roots of different grasses.

Secretion of amino acids by the roots of different plants

	Ala- mine	Amino- butyric acid	Val- ine	Leu- cine	Ser- ine	Aspar- tic acid	Glut- amic acid	Tryp- to phan	To- tal	To- tal wt of roots
Half sterile:										
Trifolium + Lolium	+	-	+	++	+	+	++	=	25.0	0.27
Trifolium + Artemisia	0	-	+	+	0	+	0	-	7.5	0.17
Lolium	+	-	0	+	0	+	0	-	7.5	0.07
Completely sterile:										
Trifolium + Lolium	+	+	0	+	+	+	+	+	17.5	0.18
Trifolium + Artemisia	+	+	++	++	+	+	0	+	27.5	0.74
Lolium + Artemisia	+	+	0	+	+	+	0	-	12.5	0.13

H.F. Linskens and R. Knapp: "Über die Ausscheidung von Amino-Säuren in reinen und gemischten Beständen verschiedener Pflanzenarten. *Planta* 45, 106-117 (1955).

It is interesting to remark that in this case tryptophane is found. It could never be detected by hydrolysis of soils or humic acids, even when the conditions had been changed, such as by hydrolysis in alkaline or reducing media.

In another case, P. Martin (Qualitative and quantitative Untersuchungen über die ausscheidung organischer Verbindungen aus den Keimwurzeln des Hafers (*Avena sativa* L.) *Naturwiss.*, 43, 227 (1956)) cultivated oats in sterile mediums and determined the substances secreted by the roots. He found with the aid of paper chromatography different amino acids such as leucine, valine, β -amino-butyric acid, glutamine, alanine, asparagine, serine, glutamic acid, aspartic acid and also fructose and glucose. In $2\frac{1}{2}$ days, an amount of amino acids of less than 3 micrograms per 100 plants had been secreted, fructose 75, and glucose 25 micrograms. In the same experiment the authors found also an interesting compound, 7-hydroxy-6-methoxy-coumarin, scopoletin. Later on we will speak about the content of phenols in the plants.



Very recently, H. O. Putnam and E. L. Schmidt succeeded in isolation of free amino acids in the soil. (Studies on the free amino acid fraction of soils, Soil Sci. 87, 22-27, (1959)). They extracted non-rhizosphere and rhizosphere soils in quantity of 6-8 kg with ethanol. Analyses by elution chromatography showed the occurrence of free amino acids in a range of concentration from 2 to 387 mg per kg of soil. In a non-rhizosphere soil there had been slightly more kinds and higher amounts of amino acids than in rhizosphere soils. Treatment with glucose and sodium nitrate to enhance microbiological activity in the non-rhizosphere sample resulted in a very marked increase in amino acid concentrations as compared to the untreated soils. They found the following amino acids in the three mentioned soils.

Free amino acid content of a rhizosphere and a non-rhizosphere soil as obtained by elution chromatography

Amino acid	Non-rhizosphere		Rhizosphere
	Glucose	NaNO ₃	
	µg/kg	µg/kg	µg/kg
Aspartic	42	28	13
Glutamic	163	31	20
Threonine	94	--	--
Serine	28	--	--
Proline	172	--	--
Valine	122	7	2
Leucine	232	12	2
Glycine	80	--	--
Lysine	12	24	2
Tryptophane	21	--	5
Phenylalanine	387	trace	3
Beta alanine	--	5	--
Arginine	--	14	--
Cystine	--	16	--
Histidine	--	17	trace

Hugh D. Putnam and E. L. Schmidt: Studies on the Free Amino Acid Fraction of Soils, Soil Sci. 87; 22-27 (1959).

For a long time the composition of the protein complex in the soil has been studied but only recently this work could be done with the possibility to identify the amino acids by paper chromatography.

235a Amino acids of the protein complex in soils Data

This table shows which kind of amino acids had been found in soil hydrolysates by the different authors. Especially, I will remark that tryptophane is not mentioned in this table.

Bremner was the first who has given data on the approximate ratios of amino compounds in acid hydrolysates of soil organic matter.

Ten different soils were investigated and the amino acid composition of their hydrolysates were determined with paper partition chromatography.

Proteins derived from residues of plants and animals are decomposed by microorganisms and the proteins of the microorganisms come into the soil. Therefore, it is not surprising that the soil hydrolysates contain most of the known naturally occurring amino acids. Some of them such as α, ϵ -Diaminopimelic acid comes from bacteria as Corynebacterium diphtheriae (Work, E. (1950) Nature, Lond., 165, 74.)

Tryptophan has not been detected and undergoes some destruction whether acid or alkali is used for hydrolysis. In the presence of carbohydrates its destruction by acid is complete.

Summarizing, one can say that the protein materials in different soils do not differ greatly in amino acid composition. The three Rothamsted soils in spite of their contrasting manurial and cultural histories and pH values have similar amino-acid distribution. Bremner believes "that apart from minor differences, the general similarities suggest that although soil organic matter is formed from a variety of materials under very different conditions, it tends to attain a more or less constant composition with respect to amino acids presumably through the activity of soil microorganisms."

After Bremner, other authors such as Stevenson and Sowden worked on the hydrolysis of organic matter in soil. (Sowden, F. J., 1955. Estimation of amino acids in soil hydrolysis by the Moore and Stein method. Soil Sci. 80:180-188.- Stevenson, F. J. 1954. Ion exchange chromatography of the amino acids in soil hydrolysates. Soil Sci. Soc. Amer. Proc. 18:373-377. - Stevenson, F. J. 1956a. Isolation and identification of some amino compounds in soils. Soil Sci. Soc. Amer. Proc. 20:201-204. - Stevenson, F. J. 1945b. Effect of some long-time rotations on the amino acid composition of soil. Soil Sci. Soc. Amer. Proc. 20:204-208.)

Very recently, a paper has been published by J. L. Young and J. L. Mortensen: (Soil Nitrogen Complexes: I. Chromatography of amino compounds in soil hydrolysates. Research Circular 61. Ohio Agricultural Experiment Station, Wooster, Ohio). These authors investigated different soils with different crops and rotations, (compare table 13a). They hydrolyzed the soils, autoclaving samples of 400 g air dried soil at 121° C and 15 psi for 18 hours in 500 ml of 6 N HCl acid. They worked up in the usual manner and desalted with Dowex-50 instead of Amberlite IR-120H according to Stevenson in the presence of 0.25% BRIJ-35 as a detergent. The absorbed amino acid was eluted successively with 1.5 N, 2 N, and 4 N HCl containing 0.25% of detergent. One ml fractions were continuously collected with an automatic fraction collector at a flow rate of 4 ml per hour. Concentration of ninhydrin reactive substances appearing in each fraction was determined. The different fractions have been investigated with paper chromatography and the relative amounts of amino acids determined. (Compare tables.) More than 50 ninhydrin reactive substances were

Die Aminosäuren im Proteinmaterial des Bodens

	SUZUKI (1908)	ROBINSON (1911)	SCHREINER (1910) SHOREY (1913)	LATHROP (1917)	KOJIMA (1947b)	BRENNER (1950) (1955a)	DAVIDSON (1951)	STEVENSON (1952)	PARKER (1952)	LUTWICK (1952)	SOWDEN (1953)	OKUDA und HORI (1954)	SIMONART (1954a)	STEVENSON (1954)
Glykokoll						+	+	+	+	+	+	+	+	+
Alanin	+					+	+	+	+	+	+	+	+	+
-Alanin						+			+					+
Serin						+	+	+	+		+	+	+	+
-Amino Buttersäure						+								
-Amino Buttersäure						+								+
Methionin						(+)								
Valin					+	+	+	+	+	+	+	+	+	+
Leucin	+	+		+	+	+	+	+	+	+	+	+	+	+
Isoleucin		+			+	+	+	+	+	+	+	+	+	+
Phenylalanin						+			+			+	+	+
Tyrosin				+		+	+	+	+			+	+	+
Histidin			+	+		+			+		(+)	+	+	+
Arginine			+											
Lysin			+	+		+	+	+	+	+	+	+	+	+
Asparaginsäure	+				+	+		+	+	+	+	+	+	+
Glutaminsäure						+	+	+	+	+	+	+	+	+
Glutamin													+	
Threonin						+	+	+	+	+	+	+	+	+
Prolin	+					+	+	+	+	+	+	+	+	+
Oxyprolin						+	+	+	+		(+)			+
d, -Diamino- primelinsäure						+			(+)					+

Huminsäuren
Moorböden
Böden
Böden
Böden
Böden und Fen
Huminsäuren
Böden
an Ton sorbierte Kolloide
Böden
in den Perkolaten sich
zersetzender Blätter
Verschiedene
Huminsäurefraktionen
Japanische Böden
Lignin
(Weizenstroh u.a.)
Lignin (Reisstroh)
Stets
Häufig
Selten
Verschiedene
Böden
Verschiedene Böden

Description of Soils Studied

Soil	pH	Organic matter %	Total carbon %	Total Nitrogen %	C/N
Wooster silt loam*					
9(K)	6.6	1.5	0.74	0.079	9.4
10(K)	6.4	1.4	0.74	0.079	9.4
3(L)	6.7	1.4	0.75	0.081	9.1
5(L)	6.8	1.5	0.79	0.084	9.3
Virgin**	5.2	2.3	1.15	0.108	10.6
Hoytville clay loam					
Virgin***	6.0	10.1	6.65	0.550	12.1

- * 9(K) 1915-36 Continuous vetch
1937-53 Continuous corn
1954-55 Corn, wheat
- 10(K) 1915-36 Continuous timothy-bluegrass
1937-53 Continuous corn with sweetclover catch crop
1954-55 Corn, wheat
- 3(L) 1915-35 Corn, soybean rotation
1936-50 Corn, ~~oats~~ with sweetclover catch crop
1950-53 Corn, oats with 8 tons/acre manure on corn
1954-55 Corn
- 5(L) 1915-53 Corn, oats rotation
1954-55 Corn

All plots similarly fertilized.

** Virgin Wooster silt loam - Native sod: principally bluegrass.

*** Virgin Hoytville clay loam - Forest: Burr oak, red oak, ash, elm, shagbark hickory with some bluegrass.

Relative Amounts of Amino Acids in Some Ohio Soils

Peak No.	Amino Acid	Mg. per 100 g. of soil					
		9(K)	10(K)	3(L)	5(L)	V-W	V-H
2	Cysteic acid	5	5.5	5.5	3.5	5	11
10	Aspartic acid*	25	29	27	32	43	182
11	Serine*						
12	Threonine	12	11	10	13	16	80
14	Glutamic acid	22	22	23	26	33	137
15	Methionine sulfone	3	0.8	1.8	2.0	1.1	10
21	Hydroxyproline	3	4	3	5	4	33
23	Glycine	10	10	11	10	12	80
26	Alanine	14	17	17	18	22	109
27	Beta-alanine	2	1	2	2	2	10
32	Gamma-amino-butyric acid	0.4	0.3	0.5	0.3	0.7	1.7
36	Valine	11	11	11	11	16	81
37	Methionine sulfoxide	0.5	0.8	0.5	0.6	1.0	6.4
38	Proline	8	9	9	10	12	48
42	Isoleucine	6	7	7	8	9	33
46	Leucine	6	7	6	7	9	58
45	Ornithine	6	7	8	8	5	35
50	Lysine	11	13	13	13	17	69
52	Histidine	2	0.3	0.7	0.8	3.5	13
55	Tyrosine	0.6	1.0	---	Trace	1.5	17
56	Arginine	1	1	---	1	1	12
57	Phenylalanine	5	4	4	5	7	34

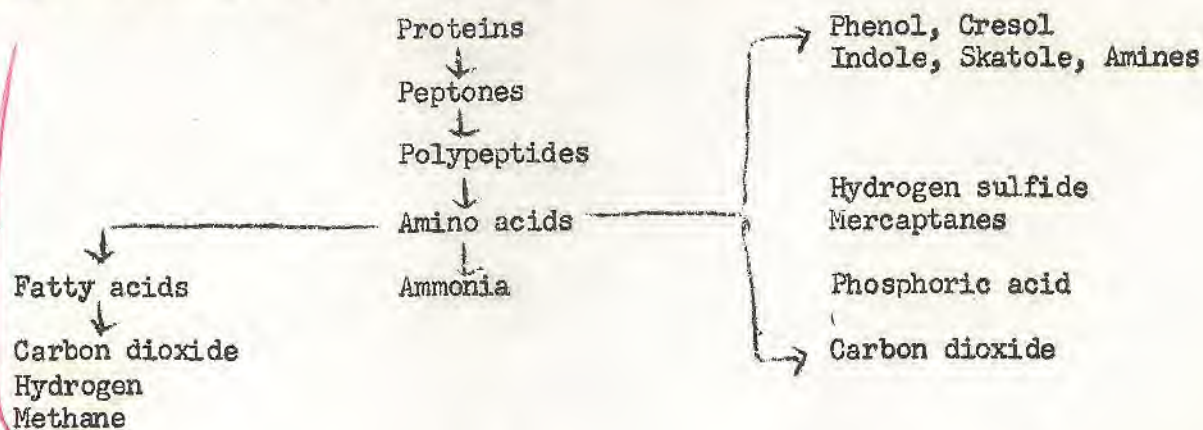
* From the spots on the paper chromatograms, these amino acids appeared to be present in roughly equivalent amounts.

detected in the desalted hydrolysates of the different soils. The results indicate that the relative distribution of the amino compounds is nearly the same in all the soils examined. The quantities of the amino acids which the different soils contain can vary considerably. The Hoytville clay loam contained larger quantities (5-8x) of amino compounds than the Wooster silt loam soils. If one calculates, however, the quantity of each amino compound present per unit weight of total carbon, no large differences can be observed.

In the next table (18) the results of the different authors are compared. It is to say that it appears that the qualitative distribution is nearly the same in every case. Of course, it is not possible to compare directly because the techniques are somewhat different in each case. The data indicates that the bulk of amino nitrogen present in soils can be accounted for by 12 to 15 compounds. The majority of ninhydrin-positive compounds detected in his study obviously must exist in minute quantities.

Most of the proteins of the soils will probably be of the living and dead organisms. Partly they are adsorbed by clay minerals. The presence of a high amount of proteins in the soils proposes stabilization of these materials. Free proteins in the soils are decomposed very easily and quickly by the various microorganisms. The amino acids decompose also very rapidly. With the exception of Putnam, Sowden and Parker (Amino nitrogen of soils and of certain fractions isolated from them. *Soil Sci.* 36, 201-208 (1953)) and also Bremner, workers have not succeeded in identifying free amino groups with 1-Fluor-2,4-Dinitrobenzene. (Bremner, J. M. Amino acids in soil. *Nature (Lond.)* 165-367 (1950)), to identify free amino acids. It can be presumed that the free amino acids found by Putnam are short lived intermediates of the decomposition of plants or microorganisms.

For our considerations about humification of residues of organisms we must discuss a survey of the decomposition of proteins (Rippel-Baldes, A.: *Grundri der Mikrobiologie*, 2. Aufl. Berlin: Springer 1952). (Waksman, S. A.: *Soil Microbiology*. New York, Wiley & Sons, 1952).



The proteins are hydrolyzed by the proteolytic enzymes to small polypeptides and to amino acids. The latter decompose enzymatically by the desaminases and amidases into ammonia and corresponding fatty acids. Under anaerobic conditions there will be formed different amines and mercaptans.

Comparison of Identified Amino Nitrogen Compounds from Acid Hydrolysates of Soil Organic Matter Fractions. Isolations Reported by Different Investigators Using Ion Exchange and Paper Chromatographic Techniques

	Relative Abundance of Amino Compounds			
	Bremner (1955)	Stevenson (1956b)	Sowden (1955)	Young & Mortensen
	Prep. IP**	Flanagan s.l.***	Lacombe soil****	Hoytville c.l.*****
	1 = weak	mg/100g soil	mg/100g soil	mg/100g soil
	10 = strong			
Cysteic acid	1	16	--	11
N-Acetyl glucosamine	--	+	--	P
Glucosamine		+	--	+
Galactosamine	<1	+	--	P
Aspartic acid	8	67	146	182
Serine	6	73	48	80
Threonine	4	81	79	137
Glutamic acid	9	94	117	10
Methionine Sulfone	<1	+	--	33
Hydroxyproline	M	+	--	80
Glycine	9	74	81	109
Alanine	9	77	46	10
β -Alanine	2	17	--	2
γ -Aminobutyric acid	2	12	--	P
α,ϵ -Diaminopimelic acid	P	10	--	P
α -Amino-n-butyric acid	1	3	--	P
Valine	8	62	58	81
Methionine Sulfoxide	<1	23	--	6
Proline	M	52	40	48
Methionine	--	+	12	--
Isoleucine		48	34	33
Leucine	10	63	39	58
Ornithine	--	9	--	35
Cystine	--	+	+	--
Lysine	3	70	--	69
Histidine	<1	7	--	13
Tyrosine	1	23	26	17
Arginine	2	17	--	12
Phenylalanine	2	24	36	34

* Symbols in table:

M = medium

P = provisional identification

+ = constituent identified but quantity not estimated

-- = not detected

** Clay loam from Rothamstead, England

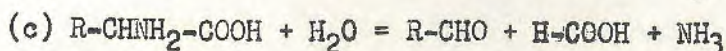
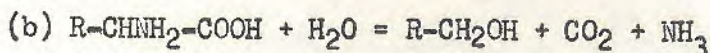
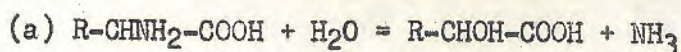
*** Brunizem - Flanagan silt loam, under grass sod, Urbana, Illinois

**** A black soil from Lacombe, Alberta, Canada

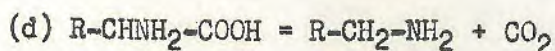
***** Clay loam from under deciduous forest, Hoytville, Ohio

The following processes go on during the decomposition of amino acids.

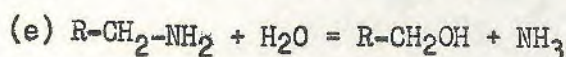
- (1) The hydrolytic de-amination form ammonia and fatty acids (a) or alcohol and CO_2 (b) or finally aldehydes and acids with lower molecular weight (c).



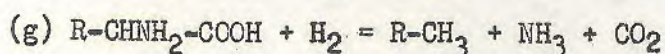
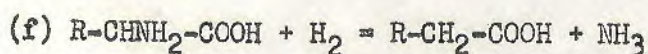
- (2) Decarboxylation forms amines



By hydrolytic de-amination of amines, alcohols are formed.



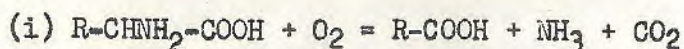
- (3) The reductive de-amination takes place only under anaerobic conditions and leads either to fatty acids (f) or to methane, methyl derivatives, respectively, (g).



- (4) The formation of ammonia is also possible without reduction under anaerobic conditions, and unsaturated fatty acids are formed.



- (5) Oxidative de-amination of amino acids occurs by aerobic organisms especially fungi. Later on we will see that model substances of low molecular humic substances are able to increase the rate of oxidative de-amination.



Microorganisms which take part in the decomposition of proteins can carry out several of these reactions.

The formation of indole derivatives during the decomposition of proteins is interesting because different indoles are growth substances such as indole-acetic acid.

Together with the decomposition of proteins, the microbial synthesis of proteins occurs. On the average, 50 to 80% of the total nitrogen of the proteins is ammonified.

Now it is the question of what happens with the part of amino acids which is not decomposed. Some of this takes part in the processes of the humification. It is not exactly known in which way. In the beginning of this lecture we mentioned that the humic substances are formed by condensation reactions among fragments of lignin and their oxidation products, and proteins or amino acids. This idea can be supported by experiments in vitro (artificial conditions).

But there are also some experiments which suggest that under different climatic conditions the amino acids react, perhaps in different ways.

J. Carles, L. Soubies and R. Gadet (Comptes rendus des seances de l'Academic des Sciences, 247, 1229-1232, seance du 20 October, 1958) investigated different types of soils, among which there had been several tropical ones from humid climates, Guinea, Cameroon and Senegal. After hydrolysis with 6 N HCl they determined the amino acids quantitatively and found that in the soils of humid tropical climate which had been rich in organic material, the following amino acids increased: (See table).

<u>Increased</u>	<u>Decreased</u>
leucine	alanine
lysine	glycocoll
phenylalanine	aspartic acid
tyrosine	glutamic acid
α, ε diamino-pimelic acid	threonine
arginine	serine
β amino-butyric acid	

The authors found almost the same results with soils fertilized with manure. The noticeable fact is that the kinds of amino acids which decrease are those from which others are formed or which are derived directly from the citric acid cycle, from the cycle of pentoses or from glycolysis.

They compared the results with those of Bremner who investigated soils from climates which are not tropic. They ascertained that the amino acids which decrease in tropical soils are more or less increased in the soils investigated by Bremner.

The authors tried to find out other relationships and made for this purpose systematic investigations. The amino acids do not vary as a function of the pH value of fertilizer or of the C/N ratio. The highest differences have been observed as being dependent upon the climate. The authors set up the hypothesis that in the presence of high amounts of organic substances and under climate conditions with high temperature and moisture there would be the possibility that among the decomposition products of organic substances are more phenolic compounds which react with the amino acids. Therefore the decrease of certain amino acids occurs. They believe that this decrease is not in connection with a special microflora. Considering the soil type red earth (comparable with the investigated soils) we mentioned that the content of humic acids were 15% and that of fulvic acids 50% of the total humus.

We found that the kind of amino acids which are decreased in the experiments of Carles and co-workers, especially form mostly dark brown products in cultures of streptomycetes when given as nitrogen source. (W. Flaig, H. Beutelspacher, E. Kuster, and G. Segler-Holzweissig, Plant and Soil IV, pp. 118-127, 1952).

	<u>Soil Fertilized with Barnyard Manure</u>	<u>Tropical Soils Humid, Rich in Organic Matter</u>	<u>Soils Investigated by Bremner</u>
leucine	+	+	
alanine	-	-	+
Valine	+		
lysine	+	+	+))
glycocoll	-	-	+
aspartic acid	-	-	+
glutamic acid	-	-	+
threonine		-	+
serine	-	-	+
β -alanine			+))
proline	+		
δ -amino-butyric acid	+	+))	
phenylalanine	+	+	
tyrosine	+	+	+))
α, ϵ -diamino-pimelic acid	+	+	+))
arginine		+	+))
hydroxyproline			
o-nithine			
amino-adipinic acid			
histidine			
histamin			

S. Carles, L. Soubiès and R. Gadet. Les acides aminés du sol et leurs variations. *Comptes rendus des Séances d'Académie des Sciences*, 247: 1229-1232 (1958).

Other products in the soil which are formed during humification, are the uronic acids. These have been determined by acid decarboxylation after the method of Lefevre and Tollens. (Lefevre, K. U., and Tollens, B.: *Ber. deut. chem. Ges.* 40, 4513 (1907)). This investigation had been made by different authors. (Shorey, E. C., and Martin, J. B.: *J. Amer. Chem. Soc.* 52, 4907 (1930) - Waksman, S. A. and Reuszer, H. W.: *Soil Sci.* 33, 135 (1932) - Springer, U.: *Z. Pflanzenernahr., Dung., Bodenkunde* 18, 129 (1940) - Bartholomew, W. V. and Norman, A. G.: *Iowa State College J. Sci.* 15, 253 (1941) - Norman, A. G. and Bartholomew, W. V.: *Soil Sci.* 56, 143, (1943) - Fuller, W. H.: *Soil Sci.* 64, 183 (1947) - Fuller, W. H., Bartholomew, W. V. and Norman, A. G.: *Soil Sci.* 64, 143 (1947)).

By boiling uronic acids with mineral acids CO_2 is split off and used for the determination of uronic acids. Shorey and Martin, and Norman and Bartholomew determined in this manner 10 to 38% of the total organic amount in soil as uronic acids. Fuller made extensive kinetic studies of the decomposition of pectin and the evolution of CO_2 in soils. After changing of the reaction conditions, he got agreeing decarboxylation curves. Therefore, it was believed that the acid decarboxylation is useful for the quantitative determination of soil uronic acids. Bremner, J. M.: (*J. Soil Sci.* 2, 67 (1951)) discussed the results of this method for the determination of uronic acids and called attention to the relatively high values. He pointed to the fact that other substances in the soil also split off CO_2 when boiled with 6 N HCl acid. By Haworth and co-workers (Haworth, W. N., Pinkard, F. W., and Stacey, M.: *Nature* 158, 836 (1946) - Forsythe, W. G. C.: *Biochem. J.* 41, 176 (1947)) and Forsyth, methods have been published to isolate polyuronides from the soil.

Deuel and co-workers (Von P. Dubach, G. Zweifel, R. Bach and H. Deuel. *Z. Pflanzenernahr., Dung., Bodenkunde* 69 (114) 97-108 (1955)) isolated some different polysaccharides out of the fraction of fulvic acids by a modification of the method of Forsyth using sorption on a mixture of four parts of active coal and one part of celite-545. He gives some results of crude polysaccharides in comparison with those of Forsyth. (See table). After removal of proteins and desalting, the yield decreases from 20 to 40%.

The purified polysaccharides were hydrolyzed and investigated with paper chromatography. In all soils, the same components could be identified: galactose, glucose, mannose, arabinose, xylose, ribose, rhamnose, uronic acids, and 2 to 3 unidentifiable components with higher R_f -values.

The content of uronic acids in the isolated polyuronides averaged 15%. The polysaccharides had an equivalent weight of nearly 1,200.

Deuel made calculations comparing the CO_2 evolved by boiling with 6 N HCl and the amount of uronic acids which are really in the soil. For instance, he determined in the Braunerde (No. 3) per 100 g soil, 420 ml CO_2 . This would correspond to a content of uronic acids of 1.68 g or 28% of the total organic matter. From this soil sample there could be isolated only 60 g of polysaccharides which form by decarboxylation only 2.2 ml. or 0.5% of the total measured CO_2 . If only polyuronides were decarboxylated in the mentioned soil there must be twelve gram polyuronide. But the total amount of organic substances was only 6 grams. Therefore, only a small part of the substances which can be decarboxylated can be polysaccharides.

To this fact Deuel gives interesting data. The Humuskarbonatboden has been decarboxylated with 20.24% HCl at 110° according to the method of Huber, G. L.: *Über die saure Dekarboxylierung von Uronsauren.* Diss. E. T. H., Zurich 1951, S. 48.

Ausbeute an „rohen Bodenpolysacchariden“

	: Prozent : organ. : Substanz	: Polysaccharide in mg pro	
		: 100 g Boden	: 100 g organ. : Substanz
Nr. 1, Braunerde	7,5	25	333
Nr. 2, Braunerde	10,0	25	250
Nr. 3, Braunerde	6,0	60	1000
Nr. 4, Humuskarbonatboden	32,5	134	412
Nr. 5, Humuskarbonatboden	20,0	19	95
Nr. 6, Erlen-Auboden	3,7	16	432
Nr. 7, Eschen-Auboden	3,0	23	766
Nr. 8, Bruchboden	9,2	74	804
Untersuchungen von Forsyth (11)			
Heather raw humus	-	-	1640
Gardener's „turf“ soil	-	-	1320
Sphagnum-cottongrass peat	-	-	1600
Pine raw humus	-	-	1940

Dubach, P. G. Zweifel, R. Bach und H. Deuel. „Untersuchungen an der Fulvo-saure-Fraktion einiger schweizerischer Böden. J. Pflanzenern. Dung. Bodenk. 69, 97 (1955).“

Decarboxylation of humus fractions of Humuskarbonatbodens (No. 5) evolved CO₂ per g ash free substance

	CO ₂ in mg.
1. Fraction of humic acids	110.2
2. Fraction of fulvic acids precipitated with alcohol (high polymeric substances)	9.7
3. Hydrophobic part of fulvic acids (ether extract)	19.8
4. Fulvic acid without 1 and 2	167.6
(5. Fractions of polysaccharides	36.6)

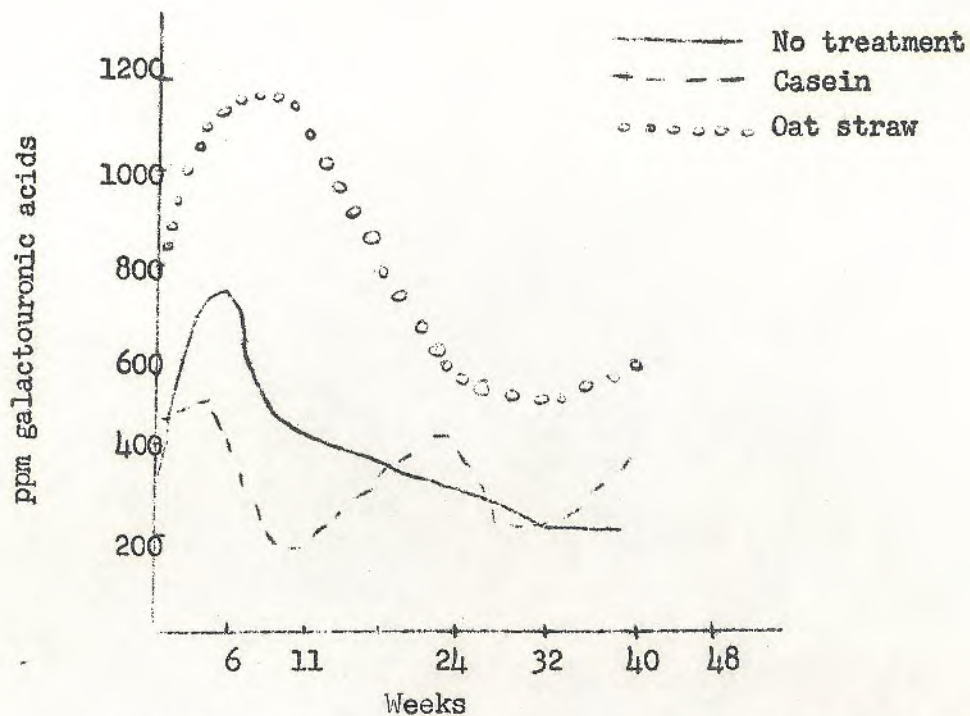
These results are interesting in so far as the kind of compounds which can easily be decarboxylated are in the fractions of humic and fulvic acids. Later on we will discuss more details.

Also in a more recent paper there has been given the method for the determination of polyuronides in soils with carbazole by Lynch, D. L., E. E. Hearn and L. J. Cotnoir, Jr.: (The determination of polyuronides in soils with carbazole. Soil Sci. Soc. of Amer. Proc. 21, No. 2 (1957)). These authors modified the method according to McComb, E. A., and McCready, R. M. (Colorimetric determination of pectic substances. Anal. Chem. 24:1630-1632. (1952) - Stark, S. M., Jr. Determination of pectic substances in cotton - colorimetric reaction with carbazole. Anal. Chem. 22:1158-1160. (1950)), for the determination of uronic acids and pectins in soils. They believe that this method was more specific, more rapid and readily reproducible, than the decarboxylation procedure.

Air dried soil samples were extracted with 0.5 N sodium hydroxide and the suspensions centrifuged. The alkaline solutions were acidified to pH 1.5 to 2.0, the humic acids are precipitated and separated by centrifugation and the supernatant liquid is mixed with an alcoholic solution of carbazole after reducing the ferric ions with stannous chloride and hydrolyzing the uronides. The red colored solutions were measured with a spectrophotometer at 520 m μ . The method has a standard error of ± 4.2 .

(Table) The authors give some examples for using their method to follow the production of uronides when plant residues decompose.

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The production of uronic acids in amended soils over a period of 40 weeks

It can be seen that uronic acids are produced rather soon after the residue begins to decompose. The amounts of uronic acids reach a maximum rapidly. Also in this case, it is necessary to determine the content of uronic acids not in random samples but during a period.

Between

the carbazole method and the decarboxylation method in determining uronic acids in soils there is a difference. With the decarboxylation method the entire soil is used. With the carbazole method only the uronic acid present in the fulvic acid fraction are determined. It could be shown with paper chromatography that nearly all uronic acids are in the fulvic acid fraction; only 10% are in the humic acid fractions.

The last results show that uronic acids may be not very important for the formation of typical humic substances.