Biochemistry of Soil Organic Matter in Relation to Crop Production

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Content

Lecture 10.

Use of Isotopes in Soil Organic Matter Studies

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Introduction

The vegetation is composed by a large number of very different compounds. Many reactions occur during the transformation of plant material in humic substances, which cause a larger variability of chemical compounds. In the past it seemed to be hopeless to elucidate all the reactions and the formed compounds. In more recent time the use of isotopes has been a useful tool for the studies of soil organic matter.

To study the decomposition of plant material in the soil and the formation of humic substances with isotopes it is necessary to produce labelled plants.

1. Production of uniformly labelled plant material.

The organic substances in soil are mainly derived from dead plants. Their decomposition is a biological process, whereby the microorganisms play an important role. For the experimental studies of the decomposition of plant material in the soil and the formation of humic substances labelled plants are used. The labelling of plants must be done by the growth during the vegetation in an atmospere of $^{14}CO_2$ in special growth chambers, that all of its constituents are uniformly labelled.

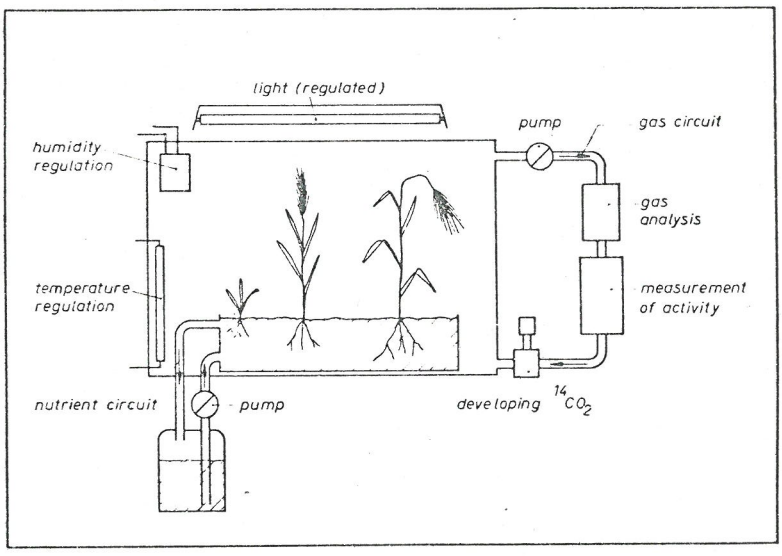

Fig. 1: Principles of the equipment of a growth chamber.

The growth chamber is a closed system. The continuous gas circuit is automatically analysed for its composition and radioactivity. The amount of added labelled carbondioxide can be regulated. Humidity and temperature are controlled and regulated with usual equipments imitating the daily rythm of the outside temperature. The same can be done with the light. The plants are grown in baskets, filled with gravels or in sand cultures. The media for growth are flooded intermittantly with nutrient solution in a rythm within fairly wide limits. The nutrient solution is stored in a tank outside the growth chamber and is circulated by a pump. In many cases the air pressure in the growth chamber is kept among 6 to 20mm of water below the pressure outside to avoid contamination of the environment with $^{14}CO_2$. In such types of growth chambers the plant can grow 6 months for instance till to the maturity of cereals.

These are only the principles of a growth chamber. Fully controlled and automatically regulated growth chambers are complicated apparatus. Since 1950 many of those growth chambers have been in operation.

One of the most successful is this of Zeller, at the Federal Institute of Agricultural Chemistry, Vienna (Austria) (ZELLER et al. 1966, 1968). He fed a cow with the labelled plants and studied the humification of the stable manure.

Some growth chambers are not fully automatically working for all conditions.

SCHARPENSEEL (1961) studied in his growth chamber the amount of activity, which must be introduced in the plant material for the studies of decomposition in soils. According to his experiments it is necessary to label plants with at least 10 µ C/g dry matter to determine the distribution of carbon-14 in the formed humic substances after rotting. For more

complicated separation processes it is necessary to use a specific activity of 30 - 100 and more μ C/g.

For this reason experiments were made by SAUERBECK (1960) and SAUERBECK and FÜHR (1966) to show, at which specific activity during labelling plant injuries of growth can be observed. They found that rye plants with an activity of 30 μ C/g carbon grow well. With 300 μ C/g carbon the rye plants show already serious injuries. Wheat plants are more resistant but their growth is also inhibited in a range of an activity of 100 μ C/g carbon.

A simpler apparatus to grow plants in a $^{14}CO_2$-atmosphere was used by RUHEMANN (1964) in our laboratory.

With this apparatus the assimilation of $^{14}CO_2$ was only for a short time. For experiments to rot plant materials it has to be taken into consideration, if plants were grown for a short or a long time in a $^{14}CO_2$-atmosphere. Only during long time growth in the CO_2- atmosphere plants were uniformly labelled in all carbon atoms. RUHEMANN (1964) showed in his short growth experiments, that in the lignin not all carbon atoms were uniformly labelled; the methoxyl groups were higher labelled than the other carbon atoms.

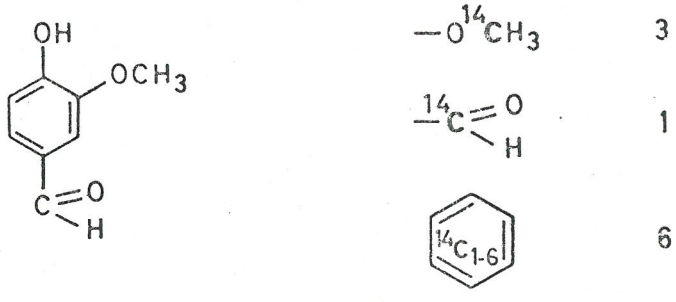

Fig. 2: Ratio of activity in vanillin from lignin of a short time experiment.

For instance vanillin was isolated after oxidation of the spruce lignin with sodium-m-nitrobenzene-sulfonate in alkaline solution. The distribution of the activity in the carbon atoms of the carbonyl-, methoxyl group and in the 6 carbon atoms of the aromatic ring has been determined as 1:3:6 this means, that the activity of the carbon atom of the methoxyl group is three times higher than in the carbon atom of the carbonyl group or on average the same in one of the carbon atoms of the aromatic ring.

These observations are important for experiments dealing with the so-called "priming action" which means a change in the decomposition rate of the original soil organic matter by addition of fresh plant material or manure. Only by the use of statistically uniformly labelled plant materials conclusions are allowed for the decomposition of the unlabelled native organic matter in the soil.

2. Turnover of organic matter in soil.

Usually organic matter does not accumulate in the soil under natural conditions. After a period of time annual additions of organic material in the soil are in a balance with the annual losses of soil organic matter. Thereafter the organic matter content of a soil remains constant. This process in which losses and gains proceed simultaneously is described as turnover and may be defined as "the flux of organic material through the organic matter in a given sample of soil" (JENKINSON 1966 a).

The rate of addition and the rate of decomposition are likely, when the environmental conditions of a soil are changed by bringing a virgin soil under cultivation or by altering a system of agriculture. Ultimately a new balance between losses and gains will be reached with the organic matter in the soil at a new equilibrium level.

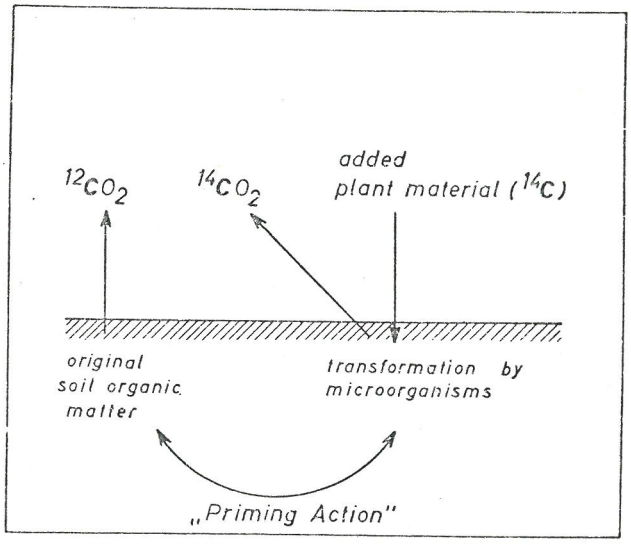

Fig. 3: Balance between increase and degradation of soil organic matter.

By addition of uniformly labelled plant material with carbon-14 the formation and the decomposition of organic material especially of humic substances have been investigated. By measuring the labelled carbondioxide in comparison with the unlabelled not only the transformation of the added plant material by the action of microorganisms could be followed during a longer period of time, but also the simultaneous decomposition of the organic matter, which has been in the soil before. The turnover of organic matter in the soil depends upon the climatic conditions, the system of agriculture, the plant associations, the pH-values and the mechanical composition of the soil.

Thus many factors are included in the processes of turnover of organic matter in soil and consequently in the formation of humic substances.

JENKINSON (1966a) has given a summary of these problems. He reports also a mathematical model for the turnover of soil organic matter:

$$\frac{dX}{dt} = A - rX \qquad (1)$$

$$X = \frac{A}{r} + \left(X_o - \frac{A}{r}\right) e^{-r} \qquad (2)$$

Assuming that all parts of X are equally decomposable, then if r is the fraction of X decomposed per year, A the annual addition of organic matter to unit area of soil sampled to depth d and t time.

SAUERBECK (1968b) investigated the stability of recently formed humic fractions in soil. He incubated labelled straw for 50 days with a low carbon soil and isolated the humic fractions with 0.1 N NaOH. The decomposition of each fraction was followed in soil for 120 days.

Non-dialysed fulvic acids lost 42 % of their carbon content, hymatomelanic acids 20 %, gray humic acids about 6 and brown humic acids about 2 %. From these experiments it can be concluded, that also the freshly formed humic acids formed by the decomposition of plant material in the soil are relatively stable products, whilst the fulvic acids undergo a quicker transformation. Furthermore FÜHR and SAUERBECK (1968) investigated the decomposition of wheat straw in the field as influenced by cropping and rotation. After one year about 55 % radio carbon could be extracted according to the method of Tyurin.

The interrelations between decomposition of various labelled plant residues and loss of soil organic matter have also been investigated by J.H. SMITH (1966). He comes to similar results as the other authors and suggests, that the protective effect on indigenous organic matter sometimes observed is caused by substances, that are in the plant material and toxic to the soil microorganisms. By these substances the

decomposition of added plant material would be limited for a time and also the rate of decomposition of the indigenous soil organic matter would be reduced.

With other investigations it could be shown with ^{14}C-labelled humic acids by MAYAUDON and SIMONART (1960) that humic acids flocculated with calcium ions are very much stabilized against microbial attack in the soil, whilst an addition of protein to the not flocculated humic acids increases the decomposition rate about 50 %.

The isotopes have become an important tool for the dynamics of the fractions of soil organic matter. SCHARPENSEEL (1960a,b) labelled humic acids by means of the Wilzbach-technique with tritium. By labelling with tritium and carbon-14 it is possible to show the transformation of one humic acid fraction into another one. Another way for synthesis of ^{3}H-labelled humic acids is to rot plant material which is added to a soil moistened with ^{3}H-labelled water. Also model humic acids have been made from tritiated purpurogalline by oxidation in the presence of a phenoloxydase.SCHARPENSEEL and BECKMANN (1964) add ^{14}C-labelled humic acids to soils and investigate the release of carbondioxide under different conditions. In a summarizing paper SCHARPENSEEL (1966a) discusses the different possibilities of labelling and the distribution of labelled material in soil organic matter; he reports 70 papers and makes new proposals for this purpose.

3. Participation of the different plant constituents on the formation of humic substances.

The decomposition of uniformly labelled plant material in the soil gives only an idea about the extent of its participation on the formation of soil organic matter, but does not allow to estimate the

importance of the single plant constituent for the formation of humic substances. Therefore it is necessary for the elucidation of the formation and for the chemical properties of humic substances to report more details especially in the field of chemistry and biochemistry.

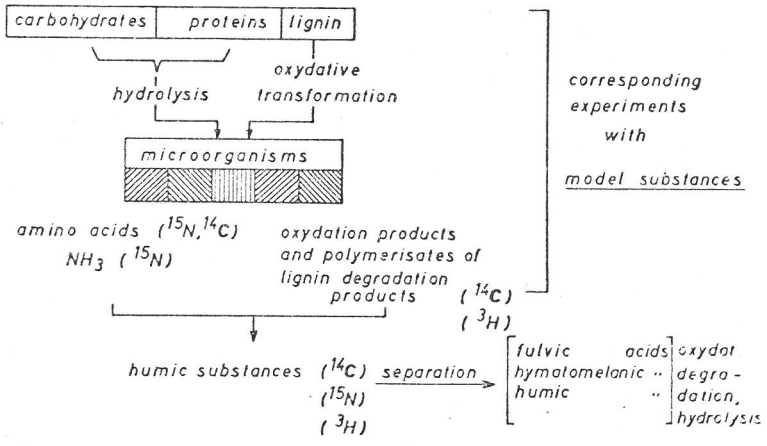

Fig. 4: Use of isotopes in soil organic matter studies.

The highmolecular plant constituents cellulose and proteins can be hydrolysed to their monomers by the enzymes of soil microorganisms, whilst lignin is not hydrolysable. So far it is known, it is degraded by oxidative processes, whereby oxidising enzymes may be involved.

The activity of the microorganisms in the soil is therefore an essential factor for the humification of plant residues.

Otherwise an extensive activity of the microorganisms can only be observed, when organic materials are in the soil to a sufficient amount for the energy supply of the microbial metabolism.

The lignin degradation products are oxidised to compounds such as phenols or quinones, which are very reactive. Both types of compounds polymerise to polymers of different composition.

By the use of plant material labelled with ^{14}C or ^{15}N labelled degradation products or by further oxidation reaction products are formed which are also labelled. Finally the formation of humic substances occurs by the reaction between the degraded lignin, the lignin degradation products, other phenolic substances synthesized by plants or microorganisms or their oxidation products, and the proteins from the plants and from the microorganisms or from other compounds formed by hydrolysis and further transformation. In this way it is possible, that labelled humic acids are formed, which are isolated and separated by the usual procedures in the fractions of fulvic, hymatomelanic and humic acids. By further oxidative or reductive degradation, hydrolysis and other reactions several informations are obtained about the chemical constitution of the isolated humus components. The different possibilities of labelling with ^{14}C, ^{15}N, ^{3}H have been used.

In many cases the use of labelled plant materials is not always sufficient. Therefore it has been necessary to make corresponding experiments with labelled model substances.

First of all the extent of the participation of the plant constituents on the formation of humic substances has been studied. (SIMONART and MAYAUDON 1958 a,b, SIMONART and MAYAUDON 1966, SIMONART, MAYAUDON and BATISTIC 1959, MAYAUDON and SIMONART 1958, 1959a,b, 1961, SØRENSEN 1963, 1966 and others).

To study the participation of carbohydrates on formation of humic substances labelled cellulose, hemicelluloses or glucose were added to soils and incubated for several days. During incubation the released carbondioxide has been absorbed in sodium hydroxide solution and measured its activity.

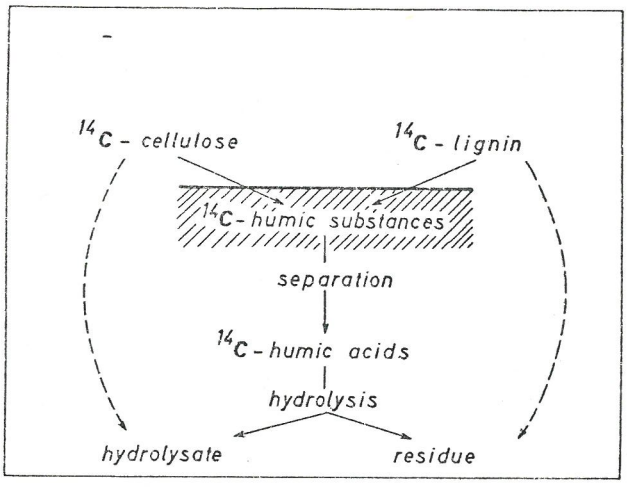

Fig. 5: Decomposition of distinct plant constituents.

Glucose is not immobilized in the soil as such. It is decomposed more rapid than hemicellulose and this more rapid than cellulose. The decomposition rate depends upon the environmental conditions used by the different authors. After the extraction and fractionation of the humic substances the humic acids have been hydrolysed. The major part of the activity has been in the hydrolysate. By paperchromatography it could be shown, that this was located in the amino acids. The carbohydrates have been transformed through the metabolism in the microorganisms to proteins, which reacted with other constituents of soil organic matter to humic acids.

On the other hand if labelled lignin is added to the soil the larger part of the activity is in the residue of the hydrolysis of the humic acids' fraction. Lignin decomposes more slowly than cellulose; this can be determined by the production of labelled carbondioxide.

Some special results about the decomposition of lignin degradation products have been reported in the lecture 2 and 3.

4. Nitrogen transformation in soil organic matter.

The cycle of the nitrogen in the system of the soil and the plant is very complicated and could not be studied in details without labelled nitrogen.

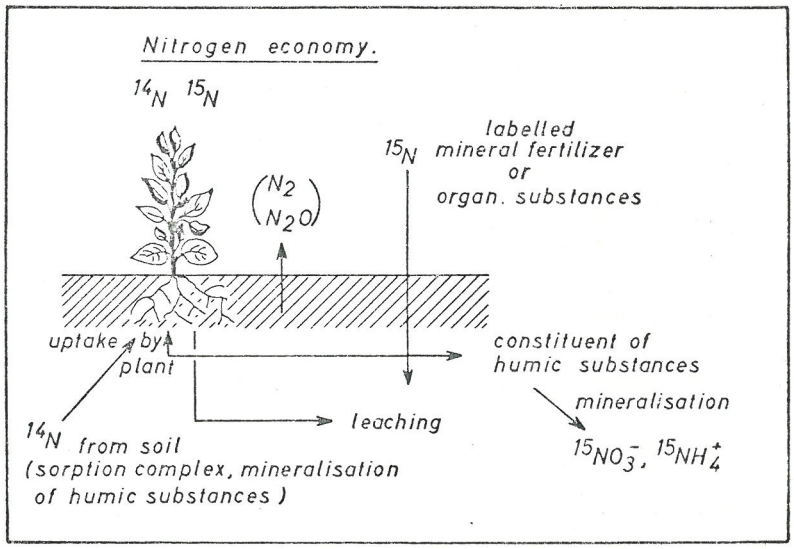

Fig. 6: Nitrogen transformation in soil organic matter.

The investigation of the nitrogen cycle can be done in different ways. The most simple way is to fertilize with ^{15}N-compounds such as ammonium nitrate or ammonium sulphate. Another way is to decompose plant material which has grown in nutrient solution with a labelled nitrogen source. By the use of labelled nitrogen salts it can be decided between the uptake of the nitrogen added as labelled mineral fertilizer and the uptake of the delivered, unlabelled nitrogen from soil organic matter by the plants. Furthermore the percentage of nitrogen can be determined which is bound in soil organic matter and mineralized again in labelled nitrate and ammonium ions. At the same time the losses by leaching as nitrate ions or by formation of gaseous compounds such as molecular nitrogen and nitrogen oxides can be followed. Also the biological fixation of nitrogen from the air by nitrogenbinding bacteria can be studied.

The first work with N^{15} on nitrogen transformation in soil was carried out about 25 years ago by NORMAN and WERKMAN (1943). Since this time many papers were published about soil-plant-relationship with heavy nitrogen. An excellent summary has been given by Sven L. JANSSON (1966 a,b) on the use of nitrogen-15 in soil organic matter studies with 50 references. Labelled plant materials and microbial tissues have been added to soil to study the mineralization of the nitrogen of soil organic matter (BROADBENT 1948, BROADBENT and NORMAN 1947, JANSSON 1958).

One of the results of this investigation is, that organic soil nitrogen may be separated in a small active fraction, which paricipated in the mineralization-immobilization turnover and a great fraction which is inerted and not included in a pathway of this cycle (JANSSON 1958). JANSSON (1963) found in long term pot experiments with labelled plant residues and microbial tissues that the main part of the nitrogen once immobilized in such substances will remain in the soils for decades.

In other investigations the nitrogen containing fraction of soil organic matter has been fractioned and the distribution of the nitrogen determined (CHENG and KURTZ 1963; STEWART, PORTER and JOHNSON 1963).

The nitrogen fixation in soil organic matter of forest soils caused by microorganisms is investigated by HÜSER (1966, 1963). Incubation experiments for four weeks in an atmosphere of air enriched with ^{15}N gas, showed that the microbial fixation of nitrogen in forest soils is limited by the availability of soil organic matter as a source of energy. HAUCK (1956, 1958, 1961, 1966) discusses nitrogen isotope distribution in nitrogen gas which is involved from soil during denitrification and its possible application to nitrogen transformations of the enriched source as well as of other nitrogen-containing material in soil.

In another paper von den HENDE (1966) describes also some experiments which deal with the incorporation of nitrogen-15 in soil organic matter. The different fixation of mineral nitrogen in the soil observed in experiments with lysimeters during 14 years is explained by the use of nitrogen-15 (GADET and SOUBIES 1962, 1966). Other experiments with lysimeters for similar problems are made by OWENS (1960). KUO and BARTHOLOMEW (1960) investigated the interchange of organic and inorganic nitrogen during the decomposition of different plants.

In interesting observation was made by BREMNER (summarized: 1968). With model experiments he found that the reaction of nitrite with soil organic matter includes the formation of aromatic nitroso compounds.

Tab. 1: Amounts of ammonium-nitrogen released from nitrogenous substances by various treatments (BREMNER and FÜHR 1966)

Substance	Ammonium released (% of total-N)		
	A	B	C
4-nitroso-phenol	18	11	3
4-nitroso-resorcinol	57	29	22
lignin-$^{15}NO_2$-product	24	8	2
peat-$^{15}NO_2$-product	42	18	-

(Treatments: A, boiling with 6 N HCl under reflux for 12 hours;
B, steam destillation with 2 N NaOH for 20 minutes
C, steam destillation with MgO for 10 minutes)

Different nitroso-phenols and the lignin and peat treated with sodium nitrite release ammonium after treatments in acidic or in alkaline medium. The amount of released ammonium is higher by acidic treatment than by alkaline.

As the nitrogen transformation in the soil is always combined with the transformation of the carbon in the soil, work is proposed with plant material labelled with N^{15} and C^{24} (JANSSON 1966a,b) The transformations

of nitrogen and carbon are closely tied together; by combined labelling with nitrogen-15 and carbon-14 many new knowledges of humus formation in the soils can be predicted. Work with ^{15}N in other directions such as in soil-plant studies is made by different authors (for instance BADZHOV and IKONOMOVA, 1971; DINCHEV and BADZHOV, Agrochim. in press and others).

5. Uptake of compounds from soil organic matter by plants.

5.1 Technical equipment for experiments with fractions of humic substances.

PRAT (1959, 1960, 1961, 1963) added labelled humic acids to water cultures of plants or on the surface of leaves and concluded that humic substances are uptaken and translocated to a small amount. According to the particle weight and size of humic acids they cannot be transported through the membran of the cell. But during the time of the experiment the humic acids are decomposed in smaller parts, which can be uptaken or transported.

We found that humic acids added to the nutrition solution in a plastic tube for dialysis have nearly the same increasing effect of the yield of dryweight of rye seedlings as the fraction as such (SÖCHTIG 1971).

PRAT does not exclude the possible uptake of labelled carbondioxide by the plants, whereby the plants may become active. The labelled carbondioxide is formed by the decomposition of the humic fractions.

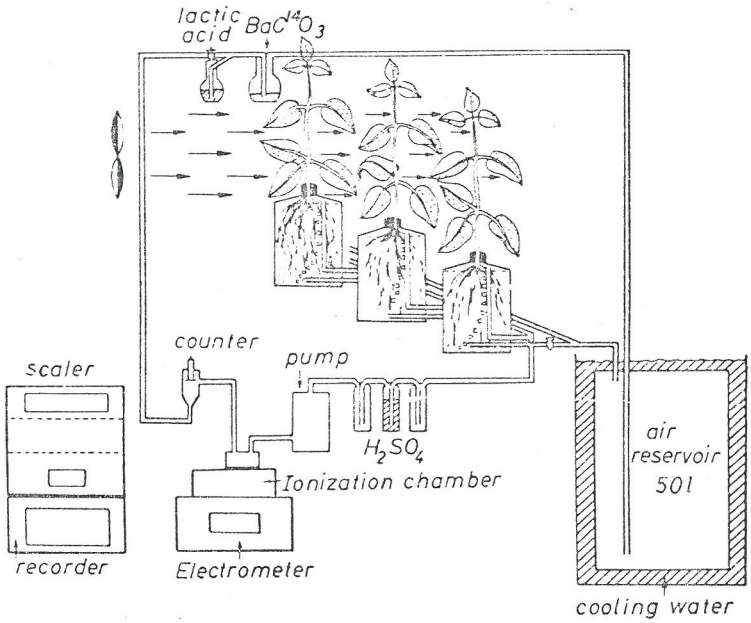

Fig. 7: Schematic diagram of $^{14}CO_2$-experiment.

FÜHR and SAUERBECK (1966) have investigated the uptake of different fractions isolated from rotted straw. They used a special experimental set-up (SAUERBECK and FÜHR 1966) and gave recommendations to exclude the CO_2-fixation by the plant roots from the decomposing material. Sunflowers as experimental plants contained between 4 % and 10 % of the applied carbon. The largest amount has been fixed in the roots, only 0.3 - 0.4 % of the water or 0.1 % of the fulvic acids were translocated into the shoots. Humic acids were adsorbed at the roots and only 0.1 % could be found in the tops. Their accumulation is therefore highly favoured in the roots. The authors obtained similar results with radish (Raphanus sativus) (FÜHR and SAUERBECK 1964) and with carrots (Daucus carota).

Furthermore the authors determined a statistically significant increase in dry-matter yield in the case of fulvic acids. In a preliminary work we found that water extracts of straw rotted during diffe-

rent times increased the dry weight of rye seedlings and the uptake of potassium (FLAIG, SAALBACH and SCHOBINGER 1960).

The effect of the water extracts is increased with its increasing methoxyl content which may be derived from lignin degradation products (MOHTADI 1962; FLAIG and MOHTADI 1965).

FÜHR and SAUERBECK (1966) worked with non-sterile cultures. To avoid all difficulties it is necessary to work with sterile cultures, especially then, when the uptake of compounds known in their chemical structure is investigated. By labelling different carbon atoms one can follow the transformation of the applied compounds, not only in the culture solution but also inside the plant.

5.2 Technical equipment for experiments with chemical compounds.

As it was necessary in our investigations about the uptake of ^{14}C-labelled phenolic acids to cultivate under absolute sterile conditions and to lead away and to absorb the carbondioxide released by the plants, the plants were cultivated in the following manner (HARMS 1967).

The sterilization of the seeds took place in a special apparatus, which was arranged round a stand and could be sterilized in the whole.

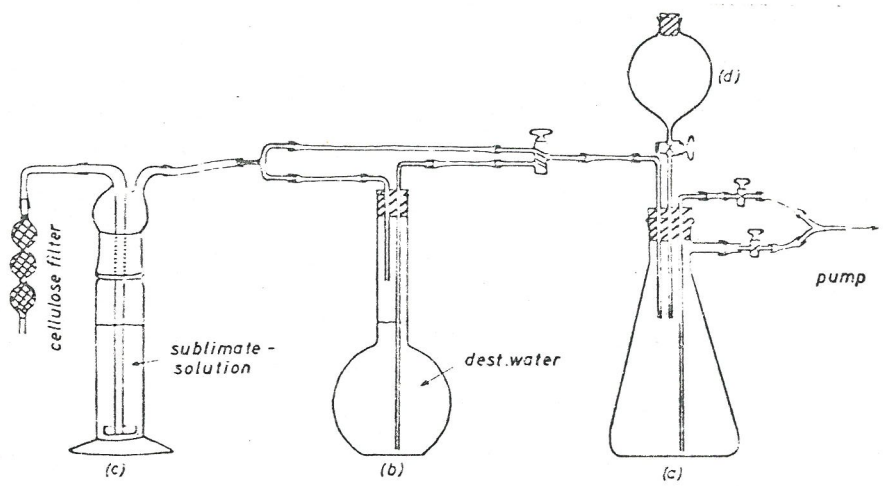

Fig. 8: Apparatus for the sterilization of the seeds.

The seed was put into a bag made of gauze and then into the filter flask. A layer of sterile water was added in order to eliminate the air under vacuum. The solution for sterilization contained 0.1 % mercury chloride and 0.1 % saponin and was allowed to run in from the apparatus (d) into the filter flask. After the mercury chloride solution has been sucked off by the glass filter pump, the seeds were washed with sterile water.

The sterilized seeds were put on Agar-plates (6 kernels per petridish) where they germinated at a temperature of 25°C degree. The used Agar, which served as a control for sterility was made of the following components: meat extract 0.3%, peptone 0.5 % and 2 % agar-agar.

When the roots of the seedlings had a length of 1 cm the seedlings were carried over into a nutrient solution under sterile conditions. The further cultivation took place in little glass tubes.

Little glass sticks hang with their curved upper part into the test tubes, while the other end of the glass stick was forked. The seedlings were put into these forked glass rods and dipped with their roots in the nutrient solution. They grew in sterilized desiccators, which were aerated with compressed air.

Before the compressed air was led into the desiccators it was led through glass bubblers filled with a solution of sulphuric acid, (normally 50 %) in order to regulate the air moisture in the desiccators with the concentration of the sulphuric acid. The exhausted air was led through a sodium hydroxide solution where the carbondioxide released by the plants was absorbed. (The scheme of the apparatus see Lecture 7).

Seven days after transplanting 1 ml of a phenolic acid solution of definite concentration was added to the nutrient solution after 3 respectively 6 days of incubation the plants were harvested, washed with destilled water, separated in sprouts and roots, pulverized in a cell-homogenizer and freeze dried. One plant of each series of tests was used for the autoradiogram.

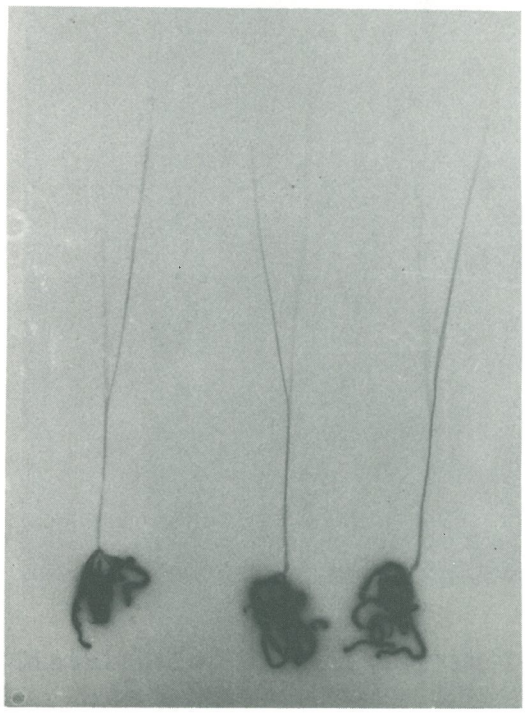

Fig. 9: Autoradiogram of wheat plants (HARMS 1967).

This picture shows the autoradiograms of plants, which have been treated with p-hydroxybenzoic acid, vanillic acid or syringic acid. It demonstrates that phenclic compounds are taken up by the roots of plants and are transported to small amounts in the leaves .

From the autoradiogram it can be seen, that the labelled compounds in the sprouts particularly are enriched in those parts of the plants which grew up during the period of incubation. The leaf venation - especially the middle nerve as the leading tissue - shows a remarkably

higher content of radioactivity.

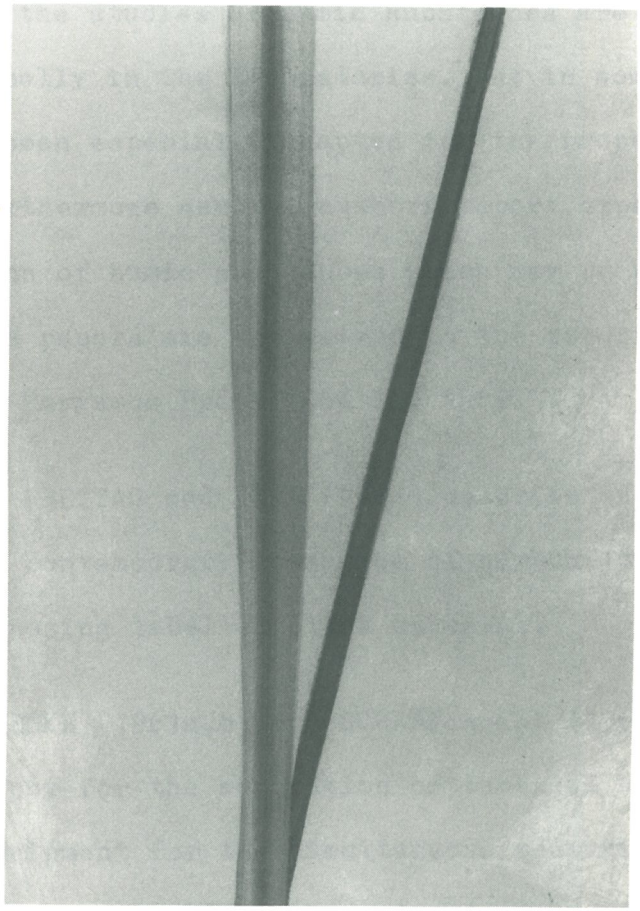

Fig. 10: Distribution of activity in leaves of weed seedlings grown before and during incubation with labelled phenolcarboxylic acids. (HARMS 1967).

For the investigations a greater number of wheat-plants has been cultivated in the described sterile culture-equipment. By differently long times of incubation (3 and 6 days) we tried to study the influence of the period of incubation and the age of the plants upon the uptake of the phenolic acids. Simultaneously the transformation products of the added compounds have been studied. 3 mg of the p-hydroxy-benzoic acid or vanillic acid or syringic acid were applied to each plant in 20 ml nutrient solution. That is nearly 1.10^{-3} molar concentration.

6. Some remarks about special experimental techniques.

The techniques for the studies of humic substances are mostly those, which are used normally in the laboratories. But in some cases different methods have been especially adapted for the properties of soil organic matter. Furthermore several authors report experiences for the work about fractions of humic substances which may be profitable to others. Most of the papers are summarized in the report of the FAO/IAEA technical meeting, Pergamon Press 1966 and 1969.

FREYTAG (1962) and FREYTAG and IGEL (1964) describe an apparatus for the continuous and contemporary measuring of carbon-14 and carbon-12 liberated by decomposing labelled plant material.

SCHARPENSEEL and MENKE (1961a,b) and SCHARPENSEEL (1966b) used radio-column chromatography for the separation of labelled humic substances and describe an equipment for the simultaneous measuring of the radioactive fractions. They use this method also to determine the purity of tritiated materials. Furthermore they investigate different adsorbents for humic substances from soil or their degradation products and different scintillators for this work of fractionation.

JENKINSON (1966c) describes the precautions for the use of carbon-14 in field work and gives many other advices. Further advices for labelling soil organic matter and its fractions are given in the already mentioned paper of SCHARPENSEEL (1966a). LITTLE (1966) informs about an apparatus for the determination of carbon-14 in soil organic matter.

Some principles of labelling and several possibilities of the use of nitrogen-15 are discussed by JANSSON (1966b). About the preparation and special microanalysis for ^{15}N-labelled material inform LAROCHE and COMBELLES (1966). According to the authors' recommendations 1 mg

of substances is sufficient for the analysis.

SCHARPENSEEL (1966c) reports about methods for tritium labelling of humic acids and precursors and gives further experimental data. Especially the possibilities with the WILZBACH-technique (1957, 1961) are discussed.

Summary

Humus research has been stimulated in different directions by the work with isotopes and many new results have been obtained. Nevertheless the use of isotopes in soil organic matter studies will expand more and more in future. About 140 years ago at the beginning of soil chemistry, at the time of THAER and SPRENGEL, from the beginning of the nineteenth century till nowadays many experiments have been made about humus with unsufficient means, whereby many errors occurred. The recent and rapid development of soil organic matter research by the use of isotopes and other modern methods let us hope, that the problems may be solved. Inorganic fertilization is only one way to increase here and to maintain there the yield of the field. But more and more we understand, why soil organic matter is an important factor for plant growth and contributes much to soil productivity.

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