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# DETERMINATION OF THE QUANTITY OF CARBON AND NITROGEN IN THE RHIZOSPHERE OF YOUNG PLANTS

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## Introduction

The concept of the rhizosphere is introduced by HILTNER in 1904 to express the zone in which the living root exerts an influence on the microbes in the soil. The chief factor of influence is the production of organic matter by the root which serves as food for the microorganisms. This organic matter consists of excretion products of the living root cells and of sloughed off and dead cells. As a result of this, the number of microorganisms in the rhizosphere, in general, will be higher than in root-free soil. The relation between both these numbers is a measure of the rhizosphere effect. According to STARKEY (1929) the rhizosphere effect depends on the type of the soil in which the roots are growing, on the species of plant and on the stage of growth of the plant.

External factors, acting on the plant or on plant and soil, also exert an influence on the rhizosphere effect i.e. light intensity and day length during the growth period (ROUATT, KATZNELSON and HENDERSON, 1960; FRENZEL, 1957), periods of dryness causing wilting phenomena followed by a rewetting of the soil (KATZNELSON, ROUATT and PAYNE, 1954), the nutrient supply of the plant and especially deficiency of certain nutrients.

The composition of the bacterial flora of the rhizosphere according to nutritional groups, as determined by LOCHHEAD and co-workers (1947, 1949, 1955), shows that the relative abundance of amino acids requiring bacteria in the rhizosphere is higher than in the soil. This may indicate an excretion of amino acids by the roots. However, in many cases a stimulation of the abundance of bacteria with simple nutritional requirements is also observed; this may point to the fact that the roots produce also general nutrients i.e. carbohydrates. It is this group of microorganisms which also is able to excrete amino acids and this in turn may give support to the development

of the group of amino acids requiring bacteria. In general, the stimulation of microorganisms in the rhizosphere points to the fact that plant roots in soil create circumstances of benefit to the development of different groups of microorganisms.

Substances which can act as a source of food for microorganisms could be demonstrated in sterile liquid or sand media in which roots were grown. In general are present varying kinds and amounts of: sugars, amino acids and other organic acids. Besides these substances some plants can excrete more specific compounds. Only few informations are available about the amount of all organic substances the roots excrete or produce in the form of sloughed off tissues during the growth. Important data are given by LYON and WILSON (1921), DEMIDENKO (1928), ROVIRA (1956) and RIVIÈRE (1961). These informations have, however, only relation to plants grown in sterile media without any sorption capacity. The absence of a microflora and of a sorption capacity of the medium gives the plant the opportunity to resorb the substances excreted once, and from the experiments of VIRTANEN et al (1933) it is known that several plants can absorb amino acids, by their roots as nitrogen nutrition. Consequently hardly anything is known about the total quantity of substances given off by the roots, especially concerning their carbon and nitrogen amount and their C/N ratio, in a structured soil with a natural sorption capacity in which the rhizosphere has a certain limitation in space.

In general the extent of the rhizosphere is insufficiently known too. STARKEY (1929) ascertained the influence of plant roots on the bacterial flora of a poor sandy soil up to 20 cm from the roots. In a recent publication PAPAVIZAS and DAVEY (1961) presented information about this subject, estimated with a set of micro borings taken from the rhizosphere of young lupins. In these small samples they determined the numbers of bacteria, fungi and actinomycetes with the plate dilution technique. The R/S values (total numbers of microorganisms in the rhizosphere/total numbers of microorganisms in non-rhizosphere soil) got in this way, showed that the rhizosphere is rather extensive and can have a radius up to 18 mm even for these young plants in these unamended soils. In amended soils the rhizosphere effect may be negligible. It may be considered as logic that the rhizosphere effect depends on the richness of microbial life in a soil. In general a fertile soil will be richer than a poor one and so the rhizosphere effect, expressed as R/S values, will be lower for a richer soil than for a poorer one.

An extensive review on this subject, offering more information is published by STARKEY (1958).

The purpose of our investigations was to get exact information about the quantities of organic matter (i.e. carbon and nitrogen) given off by the roots and about the distance of spreading of these substances from the root surface in soil.

## Material and Methods

To get this information we used a technique whereby the roots were forced to grow in a small chamber (0.5 × 4 × 8 cm) made from a thin nickel plate (0.1 mm) density perforated with punctures of 60 μ diameter. This chamber is placed in a container, and both are filled with the same substrate.

The substrate used, consists of a mixture of 70 % pure sand, 25 % finely ground K-feldspar and 5 % kaolinite with some finely granulated pumic stone to improve the structure and aeration of this artificial soil. In some experiments the amounts of feldspar and kaolinite were higher.

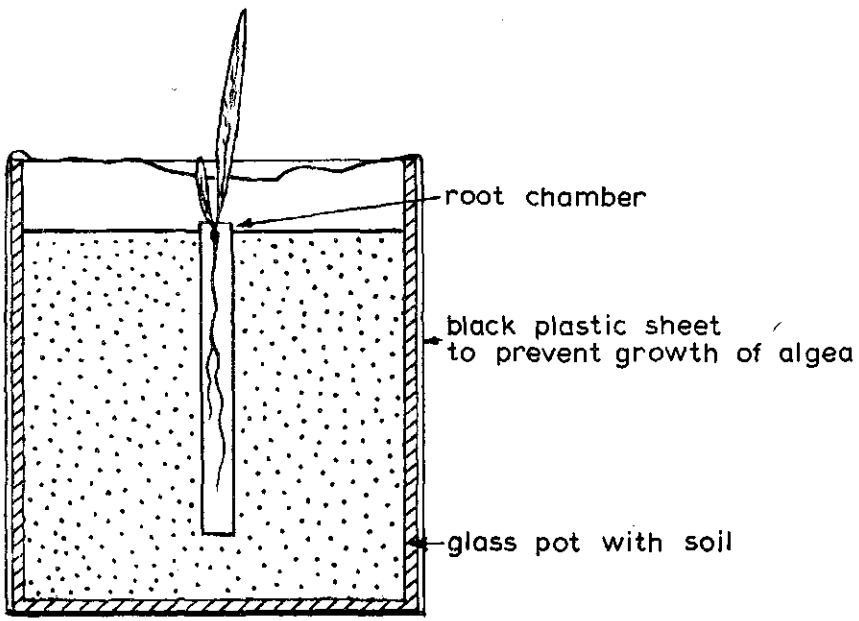



Fig. 1. Diagrammatic scheme of the experimental set up.

This substrate is very poor in organic substances, containing only 0.022 % carbon on dry basis, but it has a rather high absorption-capacity. 200 ml of a nutrient solution, containing 1.5 g of Crone's mixture per liter is added to every container, holding about 600 g of dry soil. During the experiment water is added if necessary. The nickel plate proved not to be harmful against the roots and had not a detectable oligodynamic effect upon the microorganisms of the soil. It is also inert against the substances produced by the roots and is impervious to the roots.

In the upper part of the root chamber germinated sterilized seeds are planted and allowed to grow for a required period. A diagrammatic scheme of the experimental set up is given in Fig. 1. The space within the perforated chamber becomes filled up with a mat of roots and consequently this whole space may be considered to be rhizosphere, while the space outside of the chamber still is in connection with the roots and serves as a stock of moisture and nutrients.

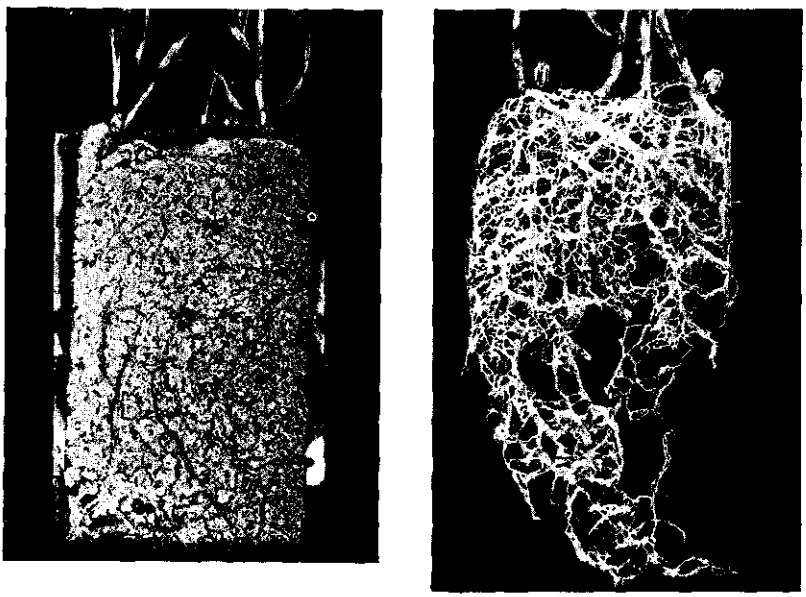
To determine the amounts of carbon and nitrogen in the rhizosphere the content of the opened root chamber (Fig. 2) is carefully washed free from soil. The washing is dried at 105° C and analyzed (fraction a). Further analyses are performed on the one centimetre thick layer of soil surrounding the root chamber (fraction b) and the rest of the soil in the container (fraction c).

The nitrogen is determined according to micro-Kjeldahl procedures; the carbon according to the dry combustion method.

## Results and Discussion

Preliminary results, obtained until now, are given in Table 1. The carbon figures are corrected for the blank (0.022 % C); the nitrogen figures could not be corrected in these experiments since $NH_4NO_3$ was added in the Crone-mixture; they therefore are less reliable.

A distinct concentration gradient for carbon from the root chamber outward is apparent in these experiments. The adapted technique consequently proved workable, but the distance of diffusion of the excreted substances proved smaller than was expected. This may be brought about by the continual flow of water and solutes towards the roots as a result of the transpiration of the plants. An other fact is that these young plants do not yet produce so much excretion products as older ones do (STARKEY, 1929).



A B

Fig. 2. A. Root chamber opened; the roots grown in it are visible.
B. The same roots, washed free from soil (The connection in the under part of the root mat got lost).

Enhancement of the sorption complex of the soil seems to lead to larger amounts of carbon preserved in the rhizosphere.

Considering the figures in Table 1 it must be kept in mind that these experiments are performed not under aseptic conditions. Part of the organic carbon given off by the roots certainly already was decomposed by the microorganisms. The data therefore represent the minimum excretion, correct figures presumably being higher.

C total refers to the sum of all carbon present in the different fractions. This value could be calculated as we knew the amounts of soil in every fraction.

With respect to the nitrogen, we already said the figures stated in Table 1 are uncertain as we do not know how much of the originally added nitrogen (0.66 mg N per 5 g of dry soil) was still present in the soil. The fact, however, that these values closely parallel those of the carbon, points to the fact that only small amounts of the mineral nitrogen might have been present. As some plants prefer nitrate nitrogen to ammoniacal nitrogen, the latter might have persisted in the soil. It is not very probable, however,

TABLE 1. *Root excretions in mg carbon and nitrogen*

| Plants | C per 5 g dry soil | | | C total | N per 5 g dry soil | | | C/N | C/100 mg dry roots | Season |
|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | a+b+c | a | b | c | a | | |
| Wheat | 1.7 | — | — | | | | | | | winter |
| | x) 8.7 | 1.4 | 0.5 | | 0.56 | 0.10 | — | 15—16 | | spring |
| | 2.7 | 0.1 | — | | 0.15 | — | — | 14—15 | | „ |
| | | | | | | | | | | „ |
| Spinach | 2.1 | 0.1 | — | | 0.30 | 0.02 | — | 7 | | „ |
| | x) 2.6 | — | — | | 0.25 | — | — | 10—11 | | „ |
| Vetch | 1.6 | — | — | 11.8 | | | | | 2.9 | Late summer |
| | 0.7 | 0.1 | 0.2 | 7.0 | | | | | 1.6 | „ „ |
| | s) 0.6 | — | — | 3.6 | | | | | 2.2 | „ „ |
| | s) 0.7 | — | 0.1 | 5.4 | | | | | 2.6 | „ „ |

x) soil with an enhanced clay minerals content.
s) experiments under conditions as for sterile cultures.
— no differences with control values.
Wheat and spinach were grown during two months, vetch during six weeks.
In each pot four plants were grown.

that ammoniacal nitrogen still will be present in considerable amounts after a 24 hours drying at 105° C in this slightly alcaline soil (pH about 8.0). So we may suppose that the quantities of nitrogen found here closely approximate the real quantities of excretion plus eventual amounts of mineral nitrogen as such assimilated by the microorganisms.

The C/N ratios given here, only have relation to the carbon and nitrogen found in the root chamber and also may be somewhat uncertain. Further experiments with sterile and non-sterile cultures, using another source of nitrogen can give further information about the correctness of the over mentioned nitrogen figures.

Following and improving this technique, further experiments are started to get more information about the total quantities of carbon and nitrogen produced in the rhizosphere of different plants of various age, grown under sterile as well as under non-sterile conditions in soils of different composition.

It might be expected that thereby a better insight will be gained in the role played by the rhizosphere in the cycle of carbon and nitrogen in the soil, providing us also with new information about formation and desintegration of humus.

## Summary

By way of introduction, the literature concerning the quantities of carbon and nitrogen given off by plant roots and about the extent of the rhizosphere is reviewed.

In our experiments the amounts of carbon and nitrogen are determined in the rhizosphere of young plants grown in a synthetic soil with a natural sorption complex. The techniques used are described. A diffusion of organic carbon from the roots over a distance of more than 1 cm (perhaps up to 5 cm) is observed.

Varying amounts of carbon were found under non-aseptic conditions: in the root chamber from 120 up to more than 1000 ppm, in the 1 cm thick layer of soil surrounding the root chamber 20 — 280 ppm and in the rest of the soil in the container 0—40 ppm.

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## Discussion

D. A. VAN SCHREVEN: How was the water added to the pots? Is it not possible that carbonaceous and nitrogenous materials are leached out from the perforated chamber during watering?
G. W. HARMSEN: First the outer pot then the rootchamber was watered. Therefore no leaching from the inner pot to the outer pot would occur during the watering.
G. TROLLDENIER: We have attempted similar experiments with plants in water cultures. Leguminous plants excrete 3—4 mg C per 100 mg root, dry matter. Non leguminous plants excreted less than 1 mg carbon. These values are similar to those represented in the contribution. We have also cultivated plants in an atmosphere containing C14 labeled $CO_2$. Continually taken samples allow us to study the progress of excretion.
G. W. HARMSEN: The chambers of perforated nickel plate are far from ideal. They have many shortcomings but we do not know a better technique. In liquid media the excretion by plant roots can be entirely different from that in soil, because of different diffusion gradients.
G. TROLLDENIER agreed but underlined that only the amount of excretion and not the composition could be influenced.