1961

Oxygen diffusion through soil and roots measured with oxygen-18

Creighton Randall Jensen
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Agriculture Commons

Recommended Citation
https://lib.dr.iastate.edu/rtd/1946

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
This dissertation has been microfilmed exactly as received

JENSEN, Creighton Randall, 1929–
OXYGEN DIFFUSION THROUGH SOIL AND ROOTS MEASURED WITH OXYGEN-18.

Iowa State University of Science and Technology
Ph.D., 1961
Agriculture, general

University Microfilms, Inc., Ann Arbor, Michigan
OXYGEN DIFFUSION THROUGH SOIL AND ROOTS
MEASURED WITH OXYGEN-18

by

Creighton Randall Jensen

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Soil Physics

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1961
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION; OBJECTIVES</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>Diffusion of Gases in Soil</td>
<td>3</td>
</tr>
<tr>
<td>Diffusion of Gases in Plant Roots</td>
<td>5</td>
</tr>
<tr>
<td>Oxygen-18 as a Tracer for Diffusing Oxygen</td>
<td>11</td>
</tr>
<tr>
<td>PREPARATION AND ANALYSIS OF OXYGEN-18 ENRICHED AIR</td>
<td>18</td>
</tr>
<tr>
<td>General Experimental Procedure</td>
<td>18</td>
</tr>
<tr>
<td>Electrolysis Train for Oxygen-18 Enrichment of Air</td>
<td>19</td>
</tr>
<tr>
<td>Components of Train for Oxygen-18 Enrichment of Air</td>
<td>27</td>
</tr>
<tr>
<td>Procedure for Analysis of Air Samples</td>
<td>36</td>
</tr>
<tr>
<td>Interpretation of Air Sample Analyses</td>
<td>40</td>
</tr>
<tr>
<td>PROCEDURES AND RESULTS</td>
<td>45</td>
</tr>
<tr>
<td>Measurements of Oxygen Diffusion in Soils, and in Soil-Plant Systems</td>
<td>45</td>
</tr>
<tr>
<td>Formulas for reducing measurements to diffusion coefficients</td>
<td>45</td>
</tr>
<tr>
<td>Measurements in soils</td>
<td>50</td>
</tr>
<tr>
<td>Measurements in soil-plant systems</td>
<td>74</td>
</tr>
<tr>
<td>Experiment on Soil-Root Oxygen Supply</td>
<td>92</td>
</tr>
<tr>
<td>Experiment on Photosynthetic Oxygen in a Soil-Plant System</td>
<td>98</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>104</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>108</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>111</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>116</td>
</tr>
</tbody>
</table>
INTRODUCTION; OBJECTIVES

Past studies of soil aeration in relation to plant growth have been chiefly concerned with assessing the effect of soil aeration on the plant rather than the effect of the plant on soil aeration. For example, measurements of gaseous diffusion in soils have been confined to the soil alone, usually without consideration of the possible effects of growing plants. While the aeration properties of the soil by itself are important and worthy of investigation, the soil and plant together form a single system which will need to be studied eventually, if a complete knowledge of soil aeration in relation to plant growth is to be obtained.

Previous methods (with one exception to be described later) of studying gas movement in soils have involved observations of the effects of imposed changes of the partial pressure of the gas on the soil. The methods necessitate the study of simple problems. In addition, the methodology precludes consideration of errors resulting from gas consumption or evolution at the boundaries of the system considered. This preclusion may result in large errors if the gas diffusion rates are low.

The stable isotope, oxygen-18, when used as a tracer for oxygen diffusion in soil-plant systems, appears to obviate some of the above difficulties. This advantage of oxygen-18
will be described in more detail later.

A method for using oxygen-18 as a tracer for oxygen diffusion in steady state systems will be presented in this dissertation. In addition, some possibilities for use of the method were investigated. These included the application of oxygen-18 to determining effective oxygen diffusion coefficients in soils, an exploratory study of the effect of plant roots on the oxygen diffusion coefficient of soil, an experiment on the effect of roots on the soil oxygen supply, and an attempt to measure photosynthetic oxygen in a soil-root system.
REVIEW OF LITERATURE

Diffusion of Gases in Soil

Diffusion is generally regarded as the only important process by which gases move through soils under ordinary conditions. This conclusion has been reached by several investigators (Buckingham, 1904; Romell, 1923; Penman, 1940; Blake and Page, 1948; Taylor, 1949; van Bavel, 1951). Consequently, investigations of gas movement in soils have been largely concerned with evaluating the diffusion rates or diffusion coefficient of gases in soils and relating the results obtained to other soil properties, usually the porosity. These investigations have been reviewed previously. A recent review is that of Wesseling and van Wijk (1957).

All of the various methods (except Rust et al., 1957) previously used to determine gas diffusion in soils have been based upon measurements of partial pressure changes. When using partial pressure changes to determine gas diffusion in soils, one is restricted to consideration of problems having a simple geometry which readily lend themselves to mathematical analysis (for examples, see van Bavel, 1951, 1952a, 1952b, 1954). In addition, very simple assumptions must be made about the activity of the soil, that is, about the evolution or consumption of gases.

A method which did not involve partial pressure changes
of the diffusing gas was that of Rust et al. (1957). They arrived at the conclusion that a non-steady state system employing self-diffusion would be the most ideal for studying the relation of a diffusion coefficient to soil porosity. They used self-diffusion of radioactive carbon dioxide with the total carbon dioxide concentration held constant. Uncertainties were involved due to sorption of the radioactive carbon dioxide, and due to exchange with previously sorbed carbon dioxide in the soil. It appears that these uncertainties would be largely eliminated in a steady state system, since a long time may then be allowed for attainment of sorption and exchange equilibria.

A distinction must be made between the true diffusion coefficient of a medium such as soil and the effective diffusion coefficient. The effective diffusion coefficient is the true diffusion coefficient modified by any soil anisotropy and by any consumption or production of the gas as it diffuses through the soil. While the true diffusion coefficient is of basic importance, the effective diffusion coefficient, although more variable, is also important; for it is the coefficient which actually indicates the movement of gases in field soils.

The evaluation of the effect of plants on the effective diffusion coefficient of soils has hitherto been confined to the determination of the effect of various seasonal or long-
term cropping and tillage practices (Blake and Page, 1948; Raney, 1949). More attention has been given to the converse problem of determining the relation of gas diffusion rates in soil to the growth of plants in the soil. Apparently, measurement of the changes of gas diffusion rates through soil as a result of, and during, the growth of plant roots in the soil has not yet been attempted.

Diffusion of Gases in Plant Roots

There is much intercellular, air-filled space for diffusion flow in plants, particularly in vascular plants, and many observations on the development, distribution, and function of this intercellular space have been recorded in the botanical literature (Sifton, 1945, 1957). Air spaces are so common in the sporophytes of vascular plants that there is a tendency to take them for granted. Sifton could not find a report of a vascular sporophyte which did not have them. In the aquatic angiosperms, air spaces reach their greatest development, both in individual size and in combined comparative volume.

McPherson (1939) made a detailed study of the formation of large air spaces (lysigenetic lacunae) in the cortex of corn roots. He showed that these air spaces resulted from the death and collapse of cells in the cortex. Furthermore, the death of these cells could be prevented or greatly re-
tarded by providing optimum aeration of the medium surrounding the root. All of his results indicated that scarcity of oxygen caused the formation of large air spaces. Similar results were obtained with wheat, barley, and oats. Experiments with different light periods showed that photosynthetic oxygen was not transported to the roots in sufficient quantity to affect the formation of these large air spaces. He made observations both in the laboratory and the field.

In view of McPherson's results, it is surprising that anatomical examination of plant root samples has not been used as a means of assessing the aeration status of soils. Large air spaces in roots should indicate poor aeration.

The presence of a diffusion pathway, through which the oxygen required by roots is supplied from the aerial portion of the plant, has been assumed to account for the ability of rice and various marsh plants to grow in media where oxygen is present only in small amounts or is entirely lacking. The investigations in support of this assumption have been reviewed recently by Bergman (1959). To verify the assumption, Bergman's review indicates that for plants which grow in a submerged soil, it should be sufficient to show that the intercellular gas contains a concentration of oxygen approaching that within and around the aerial portions of the plant. This was demonstrated for rice by van Raalte (1940); for Cladium mariscus by Conway (1937); for Nuphar advenum,
Peltandra virginica, Pontederia cordata, Typha latifolia, Sparganium eurycarpum, and Scirpus validus by Laing (1940); and for two mangroves, Avicenna nitida and Rhizophora mangle, by Scholander et al. (1955).

Vallance and Coult (1951), in a study of the oxygen supply to roots of the buckbean Menyanthes trifoliata, a bog plant, found that when the plants were kept in darkness, with the roots in nitrogen and the stem and leaves in air, the intercellular spaces of the roots contained from 13.5 to 17.5% oxygen. When the tops of the plants were in nitrogen and the roots in air, the concentration of oxygen in the roots fell to 3.1% or less in three plants, and to 6% in a fourth one. They thought that the major part of the air supply to the roots was transported through the stellar air passages. Apparently, the internal atmosphere of the roots of this plant is almost completely separated from the medium surrounding the roots. The same is probably true for other aquatic plants and perhaps for some land plants.

Sartoris and Belcher (1949) found that sugar cane could survive long periods of flooding if the growing point of the stalks was not submerged. Plants completely submerged for several days rapidly deteriorated and died without exception.

The possibility that oxygen liberated in photosynthesis may supplement atmospheric oxygen in the aeration of roots was suggested by Cannon and Free (1925). They observed that
corn, growing in soil, appeared to be more tolerant of an oxygen deficiency during sunshine than during a preceding period of cloudiness. This observation is contrary to the observations of McPherson (1939), who found that photosynthetic oxygen transport to roots was not sufficient to prevent the death of cells in the root cortex. Cannon (1932) found also that when the roots of sunflower plants or the cut ends of excised leafy branches of apricot were placed in oxygen-free distilled water, the oxygen content of the water increased when the shoots were exposed to sunlight. There was no proof that the increased oxygen content of the water resulted from increased photosynthesis.

Evidence of the diffusion of oxygen from aerial parts of plants to the roots, even though the cortex of both roots and stems consists of cells compactly arranged, is provided by the mass flow of gases through plants. Glasstone (1942) demonstrated that in 17 species tested, air under slight pressure was able to pass through their tissues, either leaves to roots or the converse. In some plants, such as tobacco, high rates of air flow were observed with very small differences of pressure. Similar results had been obtained by others. For example, Barthelemy (1874) gave evidence of a continuous air space system in *Nelumbium* and *Nymphaea* (pond lily). By blowing air into a cut petiole he could force it out through the stomata of the leaf blade. By placing one
leaf of an intact plant under reduced pressure in a belljar full of water, he could cause a continuous stream of air to enter the stomata of the free leaves, pass down through the tissues of the plant, and emerge from the leaf in the belljar.

Brown (1947) demonstrated an outflow of oxygen, nitrogen, and hydrogen from the roots of squash (Cucurbita pepo) seedlings when the shoots were exposed to these gases. From the description of his apparatus, however, it appears that a total pressure difference of about 2 or 3 cm. of water existed between the shoots and roots in his experiments. In view of the ready flow of gases through plants under total pressure differences, which was described earlier, it appears that the observed gas flow was a result of the total pressure difference between the shoot and root (and not the "active translocation" of gases in solution which he assumed).

Stiles (1960) has reviewed the subject of diffusion of oxygen and carbon dioxide inside the plant. Movement of oxygen and carbon dioxide in plant tissue is generally assumed to obey Fick's (1855) laws of diffusion. For the liquid phase of the tissue, the diffusion coefficient is assumed to be equal to or slightly less than that in water. The diffusion coefficient of oxygen in water is about $6.2 \times 10^{-7}$ cm.$^2$/sec. at $20^\circ$C. This value does not vary greatly over the range of temperature in which biological phenomena
occur. For carbon dioxide the diffusion coefficient in water is much greater than that of oxygen because of its greater solubility. The value given is $1.93 \times 10^{-5}$ cm.$^2$/sec. at 20°C. For the gas phase of plant tissue, the diffusion coefficient is much greater than for the liquid phase. For oxygen, the diffusion coefficient is $3 \times 10^5$ times greater if the (low) solubility of oxygen in water is included. Consequently, the intercellular space system in plants renders diffusion much more rapid than it would be otherwise. The effect will depend greatly upon the arrangement as well as the volume of the intercellular space. The arrangement is so varied that no generalization is possible.

In spite of the volume of work on the general subject of diffusion in living systems, there has been only a few measurements of the coefficient of diffusion of oxygen and carbon dioxide in higher plant tissues. The measurements which have been made of the diffusion coefficient were done on excised root segments in a buffer solution. From theoretical considerations and the rates of oxygen uptake and carbon dioxide release, the diffusion coefficient could be calculated after making some simplifying assumptions. Wanner (1945) obtained the value $1.91 \times 10^{-6}$ cm.$^2$/sec. for the diffusion coefficient of oxygen in onion roots in an unstirred solution. Berry (1949) and Berry and Norris (1949) obtained the values of $5 \times 10^{-6}$ cm.$^2$/sec. at 15°C and $11 \times 10^{-6}$
cm.\(^2\)/sec. at 30°C for the oxygen diffusion coefficient in onion roots. Briggs and Robertson (1948) obtained the values 1.4 to 2.2 \(\times 10^{-5}\) cm.\(^2\)/sec. for the diffusion coefficient of carbon dioxide in carrot root disks.

As was stated earlier in this review of literature, there appears to be no published work on the diffusion of gases through a pathway including soil and roots.

**Oxygen-18 as a Tracer for Diffusing Oxygen**

The restriction of diffusion measurement methods employing partial pressure changes to simple geometries was discussed in the review of gas diffusion in soil. The use of an isotopic tracer would seem to overcome this limitation. Tagging the gas diffusing across a portion of the boundary of a system would permit tracing the diffusion flow in terms of the isotopic dilution. Since no arbitrary partial pressure changes are imposed, the system is unchanged by the measurement, except for the subtraction of small samples for analysis.

Apparently this use of an isotopic tracer for gas diffusion in soil has not been attempted previously although the procedure of Rust et al. (1957) is similar. The objective of Rust et al. was to avoid total pressure differences that would result from the mixing of different gases and to avoid the concentration dependence of the diffusion coefficient. Also, the geometry of their system was quite simple.
Fountaine (1960)* mentions the measurement of diffusion of radioactive krypton in soil in the field. This use is actually equivalent to introducing a different gas and following its movement, as has been done previously (for example, Blake and Page, 1948, used carbon disulfide). Use of a radioactive tracer has the advantage of easy detection and measurement.

In a very wet soil, the extent of surface exposed at the ends of a soil column may be comparable to the internal surface of the soil pores. As a result, a large fraction of the respiratory oxygen loss (as from soil biological and chemical reactions) will occur at the end surfaces of the column. If oxygen movement is determined from partial pressure changes, the respiratory losses at the soil surfaces may result in large errors. The errors will be greater if plant roots project from the soil surface. If oxygen-18 is used as a tracer, the tagged oxygen concentration, and hence movement, is determined, as will be shown, from the product of the ratio, oxygen-18/oxygen-16, and the total quantity of oxygen-16. Respiratory losses at the soil surface will not significantly change the ratio, oxygen-18/oxygen-16, but the total quantity of oxygen-16 will be changed by respiratory

*This work was not published in complete form due to death of the author.
losses. This oxygen-16 change, on a percentage basis, will be small. The advantage of oxygen-18 as a tracer in photosynthetic systems was pointed out by Brown and Webster (1953).

There are some possible sources of error which must be considered in contemplating the use of oxygen-18 as a tracer for oxygen diffusing in soil and plants. These possible sources are 1) the separation of the isotopic forms of oxygen when they diffuse in a porous medium such as soil, 2) the dilution of the tracer oxygen-18 by exchange with other oxygen compounds in the soil or with sorbed oxygen, and 3) the fractionation of the oxygen isotopes by respiration (and photosynthesis in some cases).

Extensive use has been made of atmolysis, or the separation of gases by diffusion, to separate isotopes (Glasstone, 1958). This process requires, however, the use of a large number of stages to achieve efficient separation of isotopes having small differences of molecular weight. The separation produced by the initial stage is quite small. Sestle and Bracken (1955) employed a simple derivation from Graham's law of diffusion to calculate the separation of two isotopes diffusing in a steady state system.

Graham's law applied to the molecular forms $^{16}O^{18}$ and $^{16}O^{16}$ is

$$D*/D = (M/M*)^{1/2} = k$$

where $D$ and $D*$ are the diffusion coefficients of $^{16}O^{18}$ and
$^{16}O^{18}$, respectively, and $M$ and $M^*$ are the molecular weights (or mass numbers) of $^{16}O^{16}$ and $^{16}O^{18}$, respectively. Using Fick's (1855) first law, Senftle and Bracken showed that the original isotope ratio, $^{16}O^{18}/^{16}O^{16}$, would become $k(0^{16}O^{18}/0^{16}O^{16})$ as a result of steady state diffusion. This separation is independent of the source concentration, time, or length of the diffusion path. For $^{16}O^{16}$ and $^{16}O^{18}$ with mass numbers 32 and 34, respectively, the value of $k$ is 0.970. This value of $k$ represents a 3% reduction in the isotope ratio; correction for this reduction may be easily made when interpreting results. Thus, the error resulting from separation in a steady state system is small, constant, and may easily be corrected, if refinement warrants it.

A search of the literature failed to reveal a report of any common substance in soil, water, or air which would exchange oxygen with molecular oxygen, or catalyze this exchange, under conditions ordinarily occurring in soils. In particular, the absence of a catalyst for the exchange of oxygen with water had to be ascertained. That this type of catalyst does not exist in soils appears, indeed, to be the case. For example, Morita and Titani (1938) found no exchange between oxygen and water vapor on amorphous SiO$_2$ below 650°C but there was exchange above 700°C. Similarly, no exchange was found on quartz, asbestos or pumice at 800°C. Mackenzie and Milner (1951) discussed the catalysis of this reaction. They
stated that no appreciable isotopic exchange takes place between oxygen and liquid water in the presence of metallic oxides, hydroxides, or organic substances. However, introduction of $\text{H}_2\text{O}_2$, or of substances capable of catalyzing the decomposition of $\text{H}_2\text{O}_2$, such as finely divided platinum or catalase, would catalyze this exchange. For lack of evidence to the contrary, the assumption will be made that isotopic exchange of molecular oxygen in soil-plant systems is limited to exchange with sorbed molecular oxygen. Confirmation of this assumption awaits future work.

The quantities of sorbed oxygen in soils do not appear to be large enough to cause an appreciable isotopic dilution in a steady state system after sufficient time has been allowed for equilibration. Runkles et al. (1958) found the quantity of oxygen sorbed by three air-dry soils to be less than 2 cc./100 g. at 25°C. Much less oxygen was sorbed in the soils when moist. The time required for sorption equilibrium in their experiments was 1½ hours. (In the present experiments 2½ hours was used.)

Lane and Dole (1956) reviewed the subject of fractionation of oxygen isotopes by respiration and photosynthesis and presented some new results to show that there exists a natural oxygen isotope cycle in which the decreased oxygen-18 concentration of photosynthetic oxygen released to the atmosphere is balanced by an increased oxygen-16 uptake in
respiration.

The respiratory and photosynthetic fractionation factors are variable, but they are, on the average, the reciprocal of each other. The largest respiratory fractionation factor measured was 1.029 for spinach leaves and the bacteria, *Aerobacter aerogenes*. The mean respiratory fractionation factor for all vegetables was 1.009; for all bacteria 1.015. Two types of forest litter gave the values 1.014 and 1.016. Fractionation factors for respiring soils were apparently not determined. However, Khitrov and Zadorojny (1960) have measured the oxygen fractionation in air from several types of soils in the field. No fractionation could be detected in most instances; where it did occur, the fractionation factor ranged from 1.0013 to 1.004. A correlation was noted between the fractionation and the moisture content of the soil.

From the preceding values for the respiratory fractionation factor, it may be assumed that the maximum possible error from this source is 3%. It should be observed that this effect is an increase of the oxygen-18 concentration and is in opposition to the 3% decrease described earlier which resulted from the separation due to diffusion through the soil. Consequently, the error from the diffusive separation is reduced by the respiratory fractionation. Another error which reduces the diffusive separation error further will be described
later. Altogether, it appears that, in a steady state soil-root system, the errors resulting from diffusive separation, respiratory fractionation, and the third error to be described later, very nearly cancel each other.
PREPARATION AND ANALYSIS OF OXYGEN-18 ENRICHED AIR

General Experimental Procedure

Steady state systems were used in all of the experiments described in this dissertation. Determinations of oxygen movement in these systems were carried out as follows. Part of the open boundary was swept with air containing oxygen enriched with $^{16}O^{18}$ while the remainder of the open boundary was swept with unenriched air. Slow, low-pressure, matched air streams were employed to reduce mass air flow to an insignificant amount. At any point in the system, the fractional contributions of the two portions of the boundary to the diffusive oxygen flow were taken as indicated by the ratio in which the $^{18}$-enriched oxygen was mixed with the unenriched oxygen.

After sufficient time had elapsed for a steady state to be approximated in the system, air samples were removed from the system for analysis with a mass spectrometer. The oxygen movement in the system could be determined from the mass ratio, $^{16}O^{18}/^{16}O^{16}$, and the total oxygen concentration at appropriate points in the system.

In these experiments, the $^{18}$-enriched oxygen was prepared from $^{18}$-enriched $D_2O$ (deuterium oxide), rather than from $^{18}$-enriched $H_2O$, because the former is cheaper.
Electrolysis Train for Oxygen-18 Enrichment of Air

The apparatus, which was specially devised and developed for this work, for producing the two matched air streams, one of which is enriched with O\textsubscript{16}O\textsubscript{18}, is shown diagrammatically in Figure 1. The operation of the apparatus, which will be called an electrolysis train, will be described as a whole first. Then detailed descriptions of the function and construction of the separate components of the train will be given.

At C, in Figure 1, a stream of compressed air enters a gas washing bottle D filled with water. The air, now partially saturated with water, passes into a Pregl gas pressure regulator F. The Pregl regulator F maintains a constant air pressure (except for small, rapid, pressure fluctuations due to the bubbling in the regulator) upon the capillary metering orifices N, as well as upon one side of the flowmeter manometers U. P is a capillary damping orifice.

The air stream is divided into two substreams before it enters the capillary orifices N. The capillary orifices N are matched so that the air flow rates through them are equal when they are in place in the train. The air flow rate through the orifices N is read directly from the levels of the flowmeter manometers U. An air flow rate of about 3.7 cc./min. was used in all of the experiments; this rate is
indicated to the nearest 0.01 cc./min. by the calibrated scales of the flowmeter manometers.

One air substream leaves the apparatus at X. It consists of air having the normal $^{16}{O}^{18}$ concentration and is nearly saturated with water vapor; this air will be called unenriched air in the remainder of this dissertation. The other air substream proceeds to T where deuterium gas, produced in the left arm J of a Hoffman type electrolysis apparatus, is added to it.

This air substream, mixed with deuterium gas, now passes into a catalyst tube S contained in a boiling water bath R. The catalyst causes the deuterium gas to react with the oxygen in the air substream to form deuterium oxide. The air flow rates have been adjusted so that most, but not all, of the oxygen in the air substream is removed by this reaction. The deuterium oxide formed by the reaction condenses in the lower condensation tube of the two condensation tubes V, both of which are Pettenkoffer tubes partially filled with water. This condensed deuterium oxide is not utilized.

Oxygen, enriched to about 2.4% $^{16}{O}^{18}$, is produced in the right arm H of the electrolysis apparatus. This enriched oxygen passes through the upper of the two condensation tubes V where any deuterium oxide vapor it contains is diluted by the water in the tube. The enriched oxygen is now combined with the oxygen-deficient air substream emerging from the
lower of the two condensation tubes V. The air substream, now containing oxygen with a greater than normal $^{16}O^{18}$ concentration and nearly saturated with water vapor, emerges from the train at W.

The two equal air streams, having nearly identical chemical composition but differing in the concentration of $^{16}O^{18}$, are continuously maintained at W and X. Evidence that these two air streams are actually identical in chemical composition, but differ in the concentration of $^{16}O^{18}$ is provided by analysis with a mass spectrometer (see Figure 2).

The mass spectra of the $^{18}$-enriched and unenriched air streams are reproduced in Figure 2. The peaks produced by the various ions in the mass spectrometer are labeled with the mass numbers of these ions. The peak heights are proportional to the quantities of the ions present in the mass spectrometer. The quantities of the ions, in turn, are proportional to the concentrations of the gases, from which they are formed, in the sample analyzed. For instance, the peaks labeled 20 and 40 are produced by ions formed from argon in the air streams.

Comparison of the two mass spectra in Figure 2 shows that they differ (except for a slight difference in the mass 18 water vapor peaks) only in the height of the mass 34 ($^{16}O^{18}$) peaks. Thus, the unenriched air and $^{18}$-enriched air samples have the same composition, except for a higher concentration
Figure 2. Mass spectra of $^{18}$O-enriched and unenriched air substreams. The peaks with numbers $14, 14.5, 15, 28, 29$ and $30$ represent nitrogen isotopes. The peaks with numbers $16, 18, 32, 33$, and $34$ represent oxygen isotopes. Number $18$ represents water and oxygen-18. Number $20$ represents argon and neon. Number $14$, carbon dioxide. The mass peaks $14, 28$ and $32$ have been reduced to $1/40$ original height in order to show them completely in the figure.
of $^{16}O^{18}$ in the $^{18}$O-enriched air.

Peaks representing carbon dioxide (mass 44) and water vapor (mass 18) are small in Figure 2 because it was necessary to largely remove these gases, prior to analysis, by passing the air samples through a liquid nitrogen cold trap.

Electrolysis of $^{18}$O-enriched $D_2O$ takes place in the electrolysis apparatus (see Figure 1). This reaction produces both the deuterium gas, which removes oxygen from an air substream which flows in the catalyst tube S, and produces the $^{16}O^{18}$-enriched oxygen which is subsequently added back to the air substream. Because of the stoichiometry of the electrolysis reaction, the same quantity of oxygen will be added back to the air substream which flows through S as was removed from the air substream flowing through S, resulting in an air substream with its oxygen content unchanged. (The removal of oxygen, which had to be added back, occurred at S. The addition occurred where the two tubes V entered into the final tube supplying air at W.)

Deuterium gas is evolved at the platinum electrode L (see Figure 1) in the left arm J of the electrolysis apparatus. $^{18}$O-enriched oxygen gas is evolved at the platinum electrode L in the right arm H of the electrolysis apparatus. The electrolyzing current is produced by a battery charger B. The ammeter E provides a measure of the rate of electrolysis. The battery charger B is connected to a 115 volt A.C. power
line through a voltage regulator A; the latter serves to maintain a constant electrolyzing current independent of the A.C. line voltage variations.

A constant-temperature (32°C) water bath M surrounds the capillary metering orifices N and the portion of the electrolysis apparatus between the platinum electrodes. The water bath M reduces the effect of temperature variation upon the electrolyzing current and the air flow rate through the orifices N.

Components of Train for Oxygen-18 Enrichment of Air

The Pregl gas pressure regulator F (obtained from the A. H. Thomas Co., Philadelphia 5, Pa.) maintains a gas pressure in its outlet tube which is determined by the water level in the regulator. The purpose of the gas washing bottle D, filled with water, is to partially saturate the incoming air stream with water vapor. Then, evaporative water loss from the Pregl regulator will be reduced, resulting in only a very small decrease of the water level over a period of several days. Small, rapid fluctuations of the gas pressure, due to bubbling in the Pregl regulator, are not transmitted across the capillary metering orifices N. Similarly, the capillary damping orifice P serves to prevent transmission of the pressure fluctuations across the flowmeter manometers U; yet, at the same time, it permits the average pressure
maintained by the regulator to register in the manometers.

The capillary metering orifices were constructed and calibrated as follows. A length of glass tubing (5 mm. outside diameter) was heated in the center and drawn into a fine capillary about 15 cm. long. The capillary was then sealed with beeswax into a length of the 5 mm. glass tubing and mounted in the calibration apparatus as shown in Figure 3.

Small pieces were successively broken from the capillary until the length of capillary giving the desired air flow rate was obtained, as indicated by the calibration apparatus. The calibration apparatus (see Figure 3) consists of a water reservoir, 4 cm. in diameter, from which water is siphoned into a burette at a rate controlled by a Pregl pinch clamp. The rising water level in the burette produces an excess air pressure, indicated by a kerosene manometer, which causes air to flow through the capillary. The Pregl clamp can be adjusted to give the desired constant flow rate of water into the burette with a resulting constant flow rate of air through the capillary. A water jacket surrounding the burette minimizes fluctuations of air pressure within the burette due to ambient temperature changes.

The rate of rise of the water in the burette is measured in terms of ml./min. with a stopwatch. This rate is taken to be equal to the rate of flow of air through the capillary, at the pressure difference indicated by the kerosene manometer.
Figure 3. Apparatus for calibration of capillary flowmeter orifices
Due to the slightly increased density of the air in the burette, resulting from the excess air pressure, the air flow rate is actually greater than the measured rate. At the excess pressure occurring in subsequent experiments, this error was about 1% of the total air flow rate. The air flow rate, indicated by the manometers U, was corrected by this amount.

After a few calibration trials, two capillaries were obtained by adjusting their lengths, which would give equal (to within 0.01 ml./min.) air flow rates when placed in the electrolysis train (but differing by about 0.06 ml./min. when not in the electrolysis train). Final calibration was performed with the capillaries in the water bath M at 32°C. Figure 4 shows the calibration curve obtained for the two capillaries finally used. Scales for the flowmeter manometers U, giving air flow rates directly in cc./min., were prepared from these calibration curves.

The fluid in the flowmeter manometers U is odorless kerosene colored with a dye, Sudan III. The density of the kerosene is 0.78 g./cc.

The constant temperature water bath M consists of a plexiglas box 12 cm. square and 4 cm. deep, filled with water. Stirring of the water bath is performed by a 60 rpm timing motor with a 10 cm. diameter, toothed, plexiglas disk attached to its shaft. Temperature control of the bath is effected by a Fenwal thermostwitch which lights a 7 watt light
Figure 4. Calibration curves for two capillary flowmeter orifices; the orifices, when installed in the flow apparatus of Figure 1, delivered equal quantities of air per unit time.
bulb, immersed in the water, whenever the water temperature falls below 32°C. The water temperature is continuously maintained at 32 ± 1°C by this arrangement. The stirring motor and temperature controls are not shown in Figure 1.

The electrolysis apparatus is a Hoffman type, "Cenco-improved form", purchased from the Central Scientific Company, Chicago 13, Illinois. The electrolytic solution is O\textsuperscript{18}-enriched deuterium oxide (D\textsubscript{2}O) containing about 7% sulfuric acid. The O\textsuperscript{18}-enriched D\textsubscript{2}O was purchased from Oak Ridge National Laboratory, Oak Ridge, Tennessee, at the price $250/1000g. The use of O\textsuperscript{18}-enriched D\textsubscript{2}O, rather than O\textsuperscript{18}-enriched H\textsubscript{2}O, was incidental and determined only by the fact that the former is less expensive. The purity of the D\textsubscript{2}O was 99.85% with the O\textsuperscript{18} enrichment equal to "8.095 x normal" or about 1.6%, according to the supplier's analysis. Dilution by the addition of the H\textsubscript{2}SO\textsubscript{4} reduced this enrichment slightly. The concentration of the H\textsubscript{2}SO\textsubscript{4} varies slightly as the D\textsubscript{2}O is consumed (by the electrolysis reaction) and periodically replenished. The volume of the electrolytic solution is about 200 ml. and less than 5 ml. of D\textsubscript{2}O were consumed in any single experimental run; hence the effect of this consumption on the concentration and conductivity of the electrolytic solution was quite small in any single run.

A greater effect on the conductivity of the electrolytic solution resulted from separation of the electrolyte from
the D₂O by the ion migration. This separation gradually reduced the conductivity during a run, resulting in a decrease of approximately 10% in the electrolyzing current. This 10% decrease did not interfere sensibly with a desired steady state condition needed at the end of a 24 hour period, even though the steady state condition was different from what it would have been without the decrease. The electrolyte solution was remixed prior to each experimental run by drawing it up into the reservoir G of the electrolysis apparatus.

When sulfuric acid is used as an electrolyte, its concentration must be low, and the electrolyzing current must be small, to avoid formation of ozone and other side reactions (Engelhardt, 1904) at the anode. When KI-starch paper was used as an indicator, there was no evidence of ozone in the oxygen flowing from the right arm J of the electrolysis apparatus. Potassium hydroxide would be a better electrolyte since ozone is not formed at all concentrations and currents. But it was found that the use of KOH solution caused the seals of the platinum electrodes L to leak, necessitating the use of H₂SO₄ as an electrolyte instead. Ozone is toxic to plants, deteriorates rubber tubing, and more importantly, upsets desired stoichiometry in the electrolysis apparatus.

For future work, the electrolysis apparatus should be redesigned so that the leads for the platinum electrodes enter the apparatus above the solution level; this would permit the
use of KOH as an electrolyte. In addition, provision could be made for continuous remixing of the electrolyte solution to prevent separation of the electrolyte.

The electrolyzing current is approximately 0.45 amp. as indicated by the D.C. ammeter E. The current is supplied by the battery charger B through the voltage regulator A. The battery charger B is a Schauer model BX2 with a D.C. output of 6.5 volts. The voltage regulator A is a Sola constant voltage transformer with a 115-volt secondary coil.

The catalyst tube S is a Hempel gas palladium tube containing about 2 g. of palladium sponge. The boiling-water bath R, by maintaining a high temperature within the catalyst tube, prevents condensation of $D_2O$ formed by the combustion of deuterium gas and oxygen. Instead, the $D_2O$ condenses in the lower condensation tube V. Condensation in the catalyst tube would interfere with the air flow if it were not prevented. The water level in the bath R is maintained by a mariotte bottle and the heat for boiling the water is provided by an immersion heater; these are not shown in Figure 1.

Rubber tubing, used to connect parts of the train containing gas with a composition different from the external air, was impregnated, microchemical rubber tubing (A. H. Thomas Co. No. 8849-H) having a 3/32 in. bore and a 5/32 in. wall. Rubber tubing connections were made as short as possible.
Procedure for Analysis of Air Samples

Isotopic analyses were performed with a model 21-620 mass spectrometer, constructed by Consolidated Electrodynamics Corporation, Pasadena, California. The instrument used has an "isotope ratio-accessory" (cat. no. 21-027) which permits reading of isotope ratios directly from the instrument. Kunze (1960) and Kunze and Kirkham (1961) have described the use of this particular instrument. The principles upon which this instrument is based have been widely described and several accounts of the subject of mass spectrometry are available. The works of Wilson, et al. (1946), Inghram and Hayden (1954), and Beynon (1960) are examples.

Air samples for isotopic analysis were collected in two types of glass sample tubes. Whenever the sample was taken from one of the air streams, a Schwartz absorption tube was used. This type of tube, which will subsequently be called a double-inlet tube, was purchased from Ace Glass Incorporated, Vineland, New Jersey. The tube is in the form of a U with a stopcock and a side arm, with a 10/30 joint, at each end.

When the sample was obtained from an experimental container, a sample tube having only one opening was used; and the sample was obtained by diffusion equilibration. This type of tube, which will subsequently be called a single-inlet tube, consisted of a glass bulb of about 13 to 15 ml.
capacity attached through a stopcock to an inner $\frac{7}{25}$ joint. In the experimental work to be described subsequently, samples were collected from systems which had been allowed to approach a steady state condition over a period of about 24 hours. Hence, it was necessary to determine the rate of diffusion equilibration for the single-inlet sample tubes to see whether they would greatly lag the system in its approach to steady state conditions.

Four single-inlet sample tubes were thoroughly flushed with nitrogen gas. One tube was then closed and the other three were left open to the air in a still room. After 40 minutes one of the open tubes was closed; after 80 minutes a second tube was closed; and after 150 minutes the last tube was closed. The oxygen concentration (as indicated by the mass 32 peak height) of the gas within the sample tubes was then determined with the mass spectrometer. The results are shown in Figure 5. It can be seen from Figure 5 that the time required for the oxygen concentration to reach one half the equilibrium concentration (the oxygen concentration of air) is about 84 minutes. After 24 hours the relative $O_2$ concentration would be 0.99998. From this result it appears that the lag of this type of sample tube would be quite small in a system approaching a steady state over a period of approximately 24 hours.

The only treatment of the air samples prior to analysis
Figure 5. Diffusion equilibration rate for single-inlet air sample tubes
with the mass spectrometer was passage through a liquid nitrogen cold trap. This treatment removes water vapor and carbon dioxide from the air, as well as other gases solidifying at a higher temperature than the temperature of the liquid nitrogen. The liquid nitrogen trap was simply a double-inlet tube immersed in liquid nitrogen. Air samples passed through this tube before entering the mass spectrometer inlet system.

Six determinations of the peak ratio, mass 34/mass 32, were made on each sample starting with a pressure of about 250 \( \mu \) Hg and ending at about 200 \( \mu \) Hg. These determinations were averaged. The standard deviation of the means was 0.09\% when expressed in terms of \( ^{18}O \)-enriched oxygen present as percent of total oxygen.

One determination of the mass 32 peak height was made when the total sample pressure had dropped to 200 \( \mu \) Hg as indicated by an ionization gauge. The standard deviation of the mass 32 peak height measurements was 0.15\% when expressed as the percent of \( ^{16}O^{16} \) in the sample.

Interpretation of Air Sample Analyses

Interpretation of measurements made with the mass spectrometer was based upon the assumption that the observed height of a mass peak is proportional to the concentration, in the gas being analyzed, of the molecules from which the
component ions of the peak are formed. Thus, the ions, 
(0^{16}0^{18})^+, (0^{16}0^{17})^+, and (0^{16}0^{16})^+ compose the mass peaks 34, 33 and 32, respectively. The height of the mass 34 peak should be proportional to the concentration of the molecule \textit{O}^{16}0^{18} in an analyzed gas.

If two gases having different concentrations of \textit{O}^{16}0^{18} are mixed, the resulting concentration of \textit{O}^{16}0^{18} in the mixture will be, as will be derived below, a linear function of the ratio of the quantities of gas mixed. Conversely, the mixing ratio can be determined if the \textit{O}^{16}0^{18} concentration in the mixture and component gases is known. According to the assumption of the proportionality of mass peak height to concentration, the peak height may be used instead of concentrations to determine mixing ratios.

Suppose a quantity \( x \) of a gas having a mass 34 \( \textit{O}^{16}0^{18} \) peak height \( \alpha \) is mixed with a quantity \( y \) of another gas having a mass 34 peak height \( \beta \), giving a mixture with a mass 34 peak height \( \gamma \). The mixing ratio, \( x/y \), may be calculated as follows: One has

\[
\alpha x + \beta y = \gamma (x + y) ,
\]

which, when solved for \( x/y \), gives

\[
x/y = \frac{\gamma - \beta}{\alpha - \gamma} .
\]

In practice, with the mass spectrometer, the peak ratio,
mass \(^{34}/mass\ 32\), and the mass 32 \((^{16}O_{^{16}})\) peak height, can be determined with much greater precision than the mass 34 peak height. Consequently, it is better to calculate the mass 34 peak height from the formula:

\[
\text{mass }^{34\text{ peak height}} = \left(\frac{\text{mass }^{34}}{\text{mass }32}\right) \text{ mass }32\text{ peak height}
\]  

(2)

If Equation 2 is applied to Equation 1, and both gases have the same mass 32 peak height, \(\alpha\), \(\beta\), and \(\gamma\) may be taken to represent the peak ratio, mass \(^{34}/mass\ 32\), for the two gases and the mixture, respectively.

Furthermore, if the total quantity of the gas mixture \(t (=x+y)\) is known, Equation 1 may be written,

\[
\alpha x + \beta (t-x) = \gamma t
\]

Solving for \(\gamma\) yields

\[
\gamma = (\alpha - \beta)(x/t) + \beta
\]  

(3)

Equation 3 shows that the peak ratio, mass \(^{34}/mass\ 32\), of the mixture is a linear function of the fraction of the gas with peak ratio \(\alpha\) that is present.

Equation 3 was used to test the validity of the foregoing derivation and, in particular, the assumption of proportionality of peak height to concentration. Various mixtures were prepared of \(^{18}\text{O}-\text{enriched air and unenriched} \)
air produced by the electrolysis train. The method for preparing the mixtures was to collect a quantity of each gas in separate flasks by displacement of water. The flasks were then stoppered with serum bottle stoppers. Gas mixtures were made by withdrawing a total of 5 cc. per mixture of gas from the two flasks in varying proportions with a 5 cc. syringe. For each gas mixture, a volume of water equal to the volume of gas withdrawn was first injected into each flask. This largely avoided a cumulative pressure drop in the flasks as more samples were withdrawn.

The peak ratio $\gamma$ in the mixtures was determined with the mass spectrometer. The results are shown in Figure 6, where the peak ratio $\gamma$ is plotted as a function of the fraction $x/t$ of $^{18}$-enriched air (see Equation 3). A relatively good fit to the predicted straight line was obtained. The deviations from the straight line probably result, for the most part, from errors in the preparation of the gas mixtures. Consequently, it may be concluded that the foregoing assumption of proportionality of peak height to concentration, and the subsequent derivation, are valid.
Figure 6. Peak ratio $\gamma$ (mass $^{34}$/mass $^{32}$) as a function of the fraction $x/t$ of $^{18}$-enriched air present.
PROCEDURES AND RESULTS

Measurements of Oxygen Diffusion in Soils,
and in Soil-Plant Systems

Fick's first law was applied to determine the diffusion coefficient of oxygen through soil. Essentially, the experiment consisted of passing a steady stream of $^{18}$O-enriched air over the upper end of a vertical cylinder of soil and an equal stream of unenriched air over the lower end. After a sufficient period of time had elapsed for steady state conditions to prevail, the unenriched air stream was sampled after it passed over the lower soil surface. From the $^{16}O^{18}$ concentration in this sample and from the unenriched air stream flow rate, the rate of diffusion and diffusion coefficient for oxygen in the soil could be calculated.

Formulas for reducing measurements to diffusion coefficients

Fick's first law applied to steady state diffusion of a gas through a soil cylinder is

$$(dQ/dT) = DA(c_2 - c_1)/L,$$  \hspace{1cm} (4)

where $(dQ/dT)$ is the rate of movement of the gas through the soil in cc./sec., $c_2$ is the concentration of the gas at the upper end of the cylinder, and $c_1$ is the concentration at the lower end (expressed as partial volume in cc./cc.), $L$
is the length in cm. of the soil cylinder, $A$ is the cross-sectional area in cm.$^2$ of the soil cylinder, and $D$ is the effective coefficient of diffusion of the gas in the soil in cm.$^2$/sec.

Equation 4 may be solved for $D$ to give a formula for the coefficient of diffusion,

$$D = \frac{(dQ/dT) L}{A(c_2 - c_1)}$$  \hspace{1cm} (5)

The quantities $A$ and $L$ may be readily determined from the dimensions of the soil cylinder. The quantity $c_2$ is taken as the total concentration of $^{18}_0$-enriched oxygen above the soil. Because oxygen concentration may be less than that of atmospheric air (unenriched air) due to plant and soil respiration, $c_2$ is calculated from mass spectrometer measurements with the formula,

$$c_2 = \frac{0.209 \text{ (mass 32 peak height of } ^{18}_0\text{-enriched air})}{\text{mass 32 peak height of unenriched air}}$$  \hspace{1cm} (6)

The factor, 0.209, represents the oxygen concentration (in cc./cc.) assumed for the unenriched air. The mass 32 peak heights in Equation 6 are assumed to represent total oxygen concentrations. Actually, a small part of the oxygen is represented by the mass 34 peak height—2.4% of the $^{18}_0$-enriched oxygen, 0.4% of the unenriched oxygen. Consequently, a slight error in $c_2$ results from this assumption (a 2.0% negative error). This error was mentioned earlier, in the
review of literature, where it was stated that this error, together with the small error due to respiratory fractionation, very nearly cancels the error due to diffusion separation.

Determination of the quantities $c_1$ and $(dQ/dT)$ is based upon Equation 1,

$$\alpha x + \beta y = \gamma (x+y), \quad (1)$$

relating the mass $^{34}$ peak heights of two mixed gases to their original mass $^{34}$ peak heights. In Figure 7, $^{18}$-enriched oxygen diffusing through the soil cylinder is mixed with an unenriched air stream passing over the lower soil surface in the chamber B. Let $\alpha$ represent the peak ratio, mass $^{34}$/mass $^{32}$, of a quantity $x$ (the mass $^{32}$ peak height) of the $^{18}$-enriched oxygen diffusing through the soil into the chamber B in a unit time interval. Let $\beta$ represent the peak ratio, mass $^{34}$/mass $^{32}$, of a quantity $y$ (the mass $^{32}$ peak height) of unenriched oxygen flowing into chamber B at A in the same unit time interval. Then, according to Equation 1, $\gamma$ is the peak ratio, mass $^{34}$/mass $^{32}$, and $(x+y)$ is the mass $^{32}$ peak height for the oxygen that emerges at C in the unit time interval, when the system is in a steady state condition.

There are some corrections of Equation 1 which must be made for this case. First, a quantity $d$ of unenriched oxygen will diffuse from the chamber B into the soil, and second,
Figure 7. Theoretical diagram of $^{18}$-enriched air diffusion through a cylinder of soil.
a quantity $r$ of oxygen will be lost from chamber B to soil (and plant root) respiration. The revised equation 1 is

$$\alpha x + \beta (y-d-r) = \gamma (x+y-d-r) .$$

Equation 7 is written with the respiratory oxygen loss occurring before mixing of the two quantities of air. In actuality, mixing and respiration occur simultaneously. But, to make the error in the conservative direction (i.e., a smaller value for $x$), the respiration loss is assigned to the unenriched air stream. The error is small if $y$ is large compared with $r$. The quantity $(x+y-d-r)$ is the mass 32 peak height for the air emerging at C. Let $(x+y-d-r)$ be represented by $z$; then Equation 7 is

$$ \alpha - \beta )x + \beta z = \gamma z .$$

This equation is now solved for $x$.

$$x = z( \gamma - \beta )/(\alpha - \beta ) .$$

Using the value of $x$ from Equation 8, the rate of $^{18}O$-enriched oxygen diffusion out of the soil into B, $(dQ/dt)$, may be calculated from the formula,

$$(dQ/dt) = 0.209(x/y)(\text{unenriched-air flow rate}) .$$

The factor, 0.209, in Equation 9 represents the oxygen concentration (in cc./cc.) assumed for the unenriched air,
just as it did in Equation 6.

The quantity $c_1$ is calculated from the formula,

$$c_1 = 0.209(x/y).$$

(10)

The value of $x$ in Equation 10 is that given by Equation 8, in which $\gamma$ and $z$ represent the peak ratio, mass 34/mass 32, and the mass 32 peak height, respectively, of a sample taken just below the soil in chamber B of Figure 7.

**Measurements in soils**

Figure 8 is a cross-sectional diagram of the container in which experimental measurements of oxygen diffusion through soil (without the seedlings shown in Figure 8) were made. The container is a plexiglas cylinder with a 1/8-inch wall thickness and a 3-inch inside diameter. The top and bottom of the cylinder were closed by plexiglas sheets cemented to the cylinder. The cylinder was cut into two portions, an upper part and a lower part, the upper part serving as a removable cover for the lower part. The joining edges were machined so that, with the application of some Celvacene light vacuum grease, the cover could be sealed in place during experimental runs.

In the middle part of the lower part of the cylinder, moist soil was tamped to form a soil core. The space below the soil core was filled with dry quartz sand to support the
Figure 8. Experimental container for determination of steady state oxygen diffusion through soil, with and without, seedling roots
REMOVABLE COVER

OUTLET FOR O\textsuperscript{18} ENRICHED AIR

18 SEEDLINGS

INLET FOR O\textsuperscript{18} ENRICHED AIR

5 MM GLASS BEADS

HOLE FOR INSERTION OF AIR SAMPLE TUBE

SOIL

OUTLET FOR UNENRICHED AIR

83 MM INLET FOR UNENRICHED AIR
soil while it was being tamped into the cylinder. After the soil was in place, the sand in the lower part of the plexiglas cylinder was poured from the plexiglas cylinder through one of the openings below the soil. The soil core retained its form and was supported by the pressure of the plexiglas wall. The moisture content of the soil when tamped into place was about 23% by tamped volume. The soil core was originally 8.0 cm. in length but swelled to 8.3 cm. when water was added to it. A layer (300 gm.) of 5 mm.-diameter glass beads, 4 cm. deep, was placed on top of the soil. The purpose of the glass beads was to provide a medium for starting seedlings to grow into the soil core without disturbing the soil core.

Inlet and outlet holes for the $^{18}$O-enriched and unenriched air streams were drilled in the cylinder above and below the soil core at positions shown in Figure 8. The arms with outer ground joints of the double-inlet sample tubes were inserted into these holes. It was found that, if the ground joint had a heavy coat of vacuum grease, it would form a gas tight seal and the sample tube would be rigidly held in place. Figure 10 shows an experimental container set up with the air sample tubes in place. Holes were also drilled just above and just below the soil to accommodate two of the single-inlet sample tubes.

Webster silty clay loam was the soil used in all of the experiments. It was obtained from the Iowa State University
Figure 9. Experimental container for determination of steady state oxygen diffusion through saturated 500μ glass beads, with air sample tubes and air stream tubes (W and X in Figure 1), in place.

Figure 10. Experimental container for determination of steady state oxygen diffusion through soil and soil with plants, with air sample tubes and air stream tubes (W and X in Figure 1), in place.
Agronomy Farm on October 27, 1960. The soil was immediately sieved through \( \frac{1}{4} \)-inch hardware screen and thoroughly mixed. After mixing, the soil was placed in 1.5-mil polyethylene poultry bags (6 in. x 3 in. x 15 in.). The bags of soil were stored in an unheated cellar on the Agronomy Farm until used in an experiment. The soil received no further screening or other treatments before it was tamped into the plexiglas cylinders. The bulk density of the tamped soil before swelling was about 1.3 g./cc.

A weight budget was maintained for the container throughout the experiments to permit calculation of the air-filled porosity. The container was weighed daily and the amount of distilled water necessary to achieve the desired air-filled porosity was added. For these determinations the water held by the beads could be considered negligible. Water losses from the soil by evaporation (and transpiration plus guttation in later experiments with plants) were never more than a few cc./day. The fact that the air, above and below the soil core, was always nearly saturated was probably responsible for the small water losses.

The oven-dry weight of the soil core was determined after the experiment was terminated. However, the following procedure was employed to permit estimation of the oven-dry soil weight at the beginning of the experiment.

The polyethylene bag of soil to be used for the soil
core was weighed initially. After tamping the soil into the plexiglas cylinder, the bag with the remaining soil was weighed again to determine the quantity of soil used. Two soil samples were then taken for determination of moisture content by weight loss upon oven-drying. The oven-dry weight of soil in the core could then be estimated. Oven-dry soil weights estimated by this procedure agreed to within 1% with the weights determined directly at the termination of the experiments.

To calculate the air-filled porosity of the soil core, it was necessary to have the particle density of the soil in addition to the oven-dry soil weight. The particle density was determined by displacement of water by the soil under vacuum in a pycnometer bottle. Two determinations of particle density were considered sufficient because the soil had been thoroughly mixed, as described earlier. The values obtained were 2.5861 g./cc. and 2.5646 g./cc. The mean value, 2.575 g./cc., was used for all calculations of air-filled porosity for the experiments described in this dissertation.

The solid volume of the soil core was calculated by dividing the oven-dry weight of the soil by the soil particle density. The solid volume was subtracted from the total volume of the soil core (the latter was calculated from measurements of the soil cylinder dimensions) to obtain the total soil porosity.
The weight in g. (= volume in cc.) of water in the soil at any time could be calculated by subtracting the empty weight of the container (including the glass beads and plants, if any) and oven-dry weight of the soil from the total weight of the container. The air-filled soil porosity could then be calculated by subtracting the volume of soil water from the total soil porosity. Values of air-filled soil porosity which are given in this dissertation are expressed in terms of the volume fraction of the total volume of the soil core.

Measurements of oxygen diffusion through the soil alone were made first (without plants growing in the soil). The air sample tubes were inserted into the appropriate holes in the container, and the air stream tubes (W and X in Figure 1) were attached to pass the stream of $O^{18}$-enriched air above the soil core and the stream of unenriched air below the soil core. The container is shown set up for a run in Figure 10.

In all of the experiments, the outlet stopcock on the double-inlet sample tubes was closed down to give an opening of about 2 mm. This small opening did not affect the air stream flow rate but did serve to prevent back diffusion in the air stream.

It was assumed that the use of two equal air streams (W and X in Figure 1) which differed in velocity by less than 0.27% would minimize the development of a total pressure difference across the soil core and subsequent mass flow of air.
through the soil. Additional factors tending to minimize total pressure difference were the slow air stream flow rate (3.7 cc./min.), a negligible resistance to air flow through the plexiglas container and sample tubes, and the exhausting of the air streams to equal pressures (i.e., the atmosphere). It would be very difficult to prove experimentally that mass flow of air through the soil did not occur. But, perhaps the best evidence, which will be presented later, is the consistency of the experimental results and their close comparison with the diffusion measurements of previous workers using other methods.

During the experimental runs the containers were placed in a small indoor greenhouse covered with 1-mil polyethylene sheeting (see Figures 9 and 10). The greenhouse was next to a south window of the laboratory. It was 32 inches wide and 44 inches long and had a sloping top with the high end (32 inches) next to the window; the low end was 24 inches high. A bank of six 40-watt, white, fluorescent lights was mounted above the greenhouse to supplement the daylight from the window. A photocell and time switch were used to turn the light on when the sun was not shining except during the night. A minimum of 12 hours of light per day were thus provided for the experimental plants.

There was no temperature control of the greenhouse except the ordinary, thermostatically-controlled heating of the
laboratory in which the greenhouse was located. The polyethylene covering of the greenhouse reduced air movement within the greenhouse and provided a small amount of thermal insulation.

A thermograph was used to provide a record of the temperature within the greenhouse. The daily range of temperature within the greenhouse was usually within the range 20° to 30°C, but occasionally went as high as 38°C or as low as 18°C.

Ideally, gas diffusion measurements should be made at constant temperature, but constant temperature greenhouse facilities were not available for the present experiments. However, the desirability of a constant temperature might be questioned from the point of view of providing a natural environment for the experimental plants.

The variability of the greenhouse temperature did not appear to have introduced much variation into the experimental results. The variation due to temperature may have been reduced by the fact that the greenhouse temperature at the time the air samples were collected ranged only from about 22° to 32°C, most frequently being nearly 30°.

The problem of equilibration of the oxygen-18 in the sample tube for attachment to the mass spectrometer has been considered. There is also the problem of the equilibration time for the oxygen-18 as it diffuses through the soil. A
24-hour period, based on the work of Penman (1940) and a test below, was taken as sufficient.

Penman (1940) found that for carbon disulfide and acetone vapors diffusing through a soil sample little more than 1 in. thick the time required to achieve the steady state varied from 40 to 100 minutes, depending upon the geometry of the cores, type of soil, pore-space, and moisture content. In the case of a 6-in. core of New Zealand soil, with a porosity of 0.56, the time required was about 3 hours and probably would have been greater for a smaller porosity value. The soil cores used in the present experiments were 3.7 in. long. Comparison with the findings of Penman suggests that 24 hours is an adequate period of time for a steady state to be set up in a soil core of this length.

The experimental test on equilibration time was made by comparing the oxygen diffusion rate obtained for a soil core after 24 hour and 48 hour experimental runs. The air-filled porosity was 0.122 which corresponds to the slowest equilibration conditions. No change in oxygen diffusion rate was found after 48 hours as compared with the 24 hour results. The coefficient of diffusion was about $3 \times 10^{-6}$ cm.$^2$/sec.

Some further comments on equilibration will be given later.

At the end of the 24-hour period for attainment of the steady state, all stopcocks on the sample tubes were closed.
and the tubes were removed for mass spectrometric analysis of the air which they contained. The greenhouse temperature was noted and the plexiglas container was immediately weighed to permit calculation of the air-filled soil porosity.

Distilled water was then added to the soil to decrease the air-filled soil porosity somewhat and another period of steady state attainment was begun. In this way a series of oxygen diffusion rate measurements for the soil, as the air-filled soil porosity was gradually decreased by adding water, was obtained.

Values for the diffusion coefficient $D$ of oxygen in the soil were calculated using Equations 5, 6, 9 and 10. From the standard deviations of the peak ratio, mass $3^4$/mass $3^2$, and the mass $3^2$ peak height, the standard deviation of $D$ was calculated as $8 \times 10^{-6}$ cm.$^2$/sec.

The measured diffusion coefficients, as they varied with moisture content, ranged from 0 to 0.035 cm.$^2$/sec. The values of the diffusion coefficient $D$ as a function of air-filled soil porosity are shown for three different soil cores in Figures 11, 12 and 13. Each point represents a single determination for the moisture content indicated. One sees that simple, smooth curves can be drawn through the plotted values in the three figures. The diffusion coefficient became essentially zero at air-filled porosities of 0.07 to 0.12 for the three soil cores. This is in good agreement with the
Figure 11. Diffusion coefficient of oxygen in Webster silty clay loam soil as a function of the air-filled soil porosity
AIR-FILLED POROSITY (VOLUME FRACTION)

$D \times 10^3$ (CM$^2$/SEC)
Figure 12. Diffusion coefficient of oxygen in Webster silty clay loam soil as a function of the air-filled porosity
Figure 13. Diffusion coefficient of oxygen in Webster silty clay loam soil as a function of the air-filled porosity
AIR-FILLED POROSITY (VOLUME FRACTION)

\[ D \times 10^3 \text{ (CM}^2\text{/SEC)} \]
results of previous workers, as reported by Wesseling and van Wijk (1957). At air-filled porosities greater than 0.17 for Figures 11 and 12, and greater than 0.25 for Figure 13, but less than 0.30, the curves become straight lines. No oxygen diffusion measurements were made at air-filled porosities greater than 0.30.

Comparing data from the curves in Figures 11, 12 and 13, (see Table 1) one sees that the nearly equal bulk densities gave quite different diffusion coefficient determinations. These results show that the bulk density (of the oven-dry soil) should not be considered as the only factor governing the diffusion coefficient D. The method of packing was probably not the same and may be quite critical. The water distribution may have been quite different. These are factors

<table>
<thead>
<tr>
<th>Air-filled soil porosity</th>
<th>Figure from which data are taken</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B.D.=1.21 g/cc</td>
<td>0.2</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>0.10</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td></td>
<td>1.5</td>
<td>3.7</td>
<td>0.3</td>
</tr>
<tr>
<td>0.20</td>
<td></td>
<td>7.9</td>
<td>11.7</td>
<td>2.7</td>
</tr>
<tr>
<td>0.25</td>
<td></td>
<td>16.7</td>
<td>-</td>
<td>12.4</td>
</tr>
<tr>
<td>0.30</td>
<td></td>
<td>25.6</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
which might overshadow the oven-dry bulk density effect. But the purpose here is not primarily to discuss factors influencing diffusion. It is to show a new method for its measurement. The smooth curves of Figures 11, 12 and 13 indicate the measurement methodology for both the D values and porosity values were good. These latter values were a matter of laboratory weight measurements and did not introduce a problem.

In Figure 14, the results which were shown before in Figure 11 are compared with those of several previous workers. For the purpose of comparison, in Figure 14 the ratio $D/D_0$, rather than $D$, is given as a function of air-filled soil porosity. $D_0$ is the diffusion coefficient of the gas into air at the temperature and pressure at which $D$ was measured. In observing the $D/D_0$ vs. porosity values in Figure 14, both for the present work and the other data (except for the values of van Bavel and Penman), the reader should recognize that moist soil is in question and that, therefore, the well-known expression $D/D_0 = 0.6$ (porosity) is not valid. The results of other workers shown in Figure 14 were taken from Wesseling and van Wijk (1957) who presented them graphically. The solid points shown in Figure 14 represent findings of van Bavel (1952a) using alcohol, Penman (1940) using carbon disulfide and acetone vapors, Buckingham (1904) using carbon dioxide, and Taylor (1949) using oxygen.
Figure 14. Ratio of the diffusion coefficient $D$ in soil to the diffusion coefficient $D_0$ in air as a function of air-filled porosity. Comparison of present work with that of van Bavel (1952a), Penman (1940), Buckingham (1904), and Taylor (1949)
Air-filled porosity (volume fraction) vs. $(D/D_0) \times 10^2$ for different researchers:
- Van Bavel
- Penman
- Buckingham
- Taylor
- Present work

The graph shows a trend where the air-filled porosity increases with $(D/D_0) \times 10^2$, indicating a non-linear relationship.
For the (open circles) values of $D/D_0$ in Figure 14 stemming from Figure 11, a value of 0.212 cm.$^2$/sec. was used for $D_0$. This value was calculated from a standard diffusion coefficient $D_s$ for oxygen into air at standard temperature and pressure, using a reduction formula given by Roberts (1957):

$$D_0 = D_s (T_o/T_s)^n (p_s/p_o)$$

(11)

where $D_s = 0.178$ cm.$^2$/sec., $T_s = 273^\circ$K, $p_s = 760$ mm. Hg, $n = 1.75$, $T_o = 297^\circ$K and $p_o = 740$ mm. Hg. The values for $T_o$ and $p_o$ are the mean temperature and barometric pressure at the termination of the experimental runs.

The agreement of the data from Figure 11, which are shown as present work (open circles) in Figure 14, with the results of other workers (solid points) is good. The curve of Figure 11 was selected for plotting in Figure 14 because it is intermediate between the curves of Figures 12 and 13. If the curve of Figure 12 were plotted in Figure 14 it would have nearly the same slope as the curve for present work but would lie slightly to its left and still well within the range of variation of the points of Taylor. The curve of Figure 13 has a greater slope than the curve for the present work, lying to its right (and slightly beyond the range of Taylor's values) for low values of $D/D_0$ and to its left (and slightly within the range of Taylor's values) for high values of $D/D_0$. 
Measurements in soil-plant systems

The effect of plant roots upon the oxygen diffusion coefficient of the soil was investigated using the same soil cores, plus one additional soil core, as used in the oxygen diffusion measurements described in the preceding section. After the series of oxygen diffusion measurements, with decreasing air-filled soil porosity, had been completed for the soil alone, 18 pre-germinated corn seeds (var. Iowa 4570) were planted 1.5 cm. deep in the 5-mm. glass beads above the soil. The primary roots of the seedlings penetrated the soil core and emerged into the space below the soil in the plexiglas chamber (Figure 8). Measurements were made, by the same procedure used before with soils alone, of the oxygen diffusion rate through the soil during the period of several days in which primary roots continued to emerge from the bottom of the soil core.

In calculating the air-filled soil porosity by the container weight budget method, described earlier in this chapter, a correction had to be made for the weight of the seedlings. Estimations of the weight of the seedlings during the experiment were made with the formula,

\[
\text{Estimated seedling weight} = (F-I)(h/h_1) + I, \\
\]

where \( F = \text{Measured weight of seedlings at end of experiment} \).
\[ I = \text{Measured weight of pre-germinated seeds at beginning of experiment.} \]

\[ h = \text{Mean height of seedlings at time of estimation.} \]

\[ h_l = \text{Mean height of seedlings at end of experiment.} \]

Although this formula is approximate, it was considered sufficiently accurate because the seedling weight was small (less than 25 g.) in relation to the soil weight (about 470 g.).

It was found that the oxygen diffusion rate through the soil increased as the roots came through the soil. Even at low air-filled soil porosities where the oxygen diffusion rate had been too small to measure with the soil alone, the oxygen diffusion rate was increased to a measurable level when roots emerged from the lower surface of the soil into the air. In Figure 15, several measurements of the oxygen diffusion coefficient for the soil core of Figure 11 are shown, both with and without roots in the soil. Note that the ordinate scale in Figure 15 is expanded by a factor of 10 compared with the ordinate scale of Figure 11.

The increased oxygen diffusion coefficients with roots in the soil were found in four such experiments, namely, 3 soil cores with corn roots and 1 with rice roots.

The oxygen diffusion coefficients, with roots in the soil, in Figure 15 are somewhat scattered but may bear a relationship to the air-filled soil porosity as shown by the
Figure 15. Diffusion coefficient of oxygen in Webster silty clay loam, without and with corn seedling roots, as a function of air-filled soil porosity. "Number of primary roots" is the number of vertical primary roots penetrating the entire length of the soil core.
SOIL

SOIL + ROOTS

$D \times 10^4$ (CM$^2$/SEC)

AIR-FILLED POROSITY
(VOLUME FRACTION)

0

0.05

0.10

0.15

5

10

15
dashed line in Figure 15. Investigations to determine whether this relationship is essential or accidental will be discussed later.

Figure 16 shows the relationship of the diffusion coefficient of oxygen through the soil to the number of primary roots emerging from the bottom of the soil core for the four experiments conducted. The $D$ values for zero number of primary roots correspond to soil core conditions just before seeds were planted in the glass beads (with no soil disturbance).

The three curves, A, B and C, for corn, show an initial, rapid increase of the diffusion coefficient which, for curves A and C, reaches a maximum and then drops off rapidly. The decrease following the maximum coincided approximately with the formation of lateral roots on the primary roots. The decrease of measured oxygen diffusion at that time may have been the result of increased oxygen consumption associated with lateral root formation and respiration.

At the time when the oxygen diffusion coefficient for the curve A in Figure 16 had reached a maximum and began to decrease, FAA (a mixture of 50 cc. of 95% ethyl alcohol, 5 cc. glacial acetic acid, and 10 cc. formalin) was added to the soil to kill the roots without altering their structure. FAA is commonly used in microtechnique for killing and fixing plant tissues (Sass, 1958).
Figure 16. Diffusion coefficient of oxygen in Webster silty clay loam as a function of the number of primary seedling roots passing through the soil. Curves A, B and C are for corn seedling roots. Curve D is for rice (var. Caloro) seedling roots. "FAA" is a chemical mixture for killing and fixing plant tissue.
CORN

RICE

KILLED WITH FAA

FLOODED

D \times 10^4 \text{ (CM}^2/\text{SEC})

NUMBER OF PRIMARY ROOTS
As shown in Figure 16, the result of killing the roots with FAA was, for essentially the same moisture content, a large decrease of the diffusion coefficient to a value less than the initial diffusion coefficient with soil alone. However, a large quantity of gas (probably formaldehyde) was evolved when the FAA was added to the soil, and the gas, expanding and escaping from the soil, may have changed the soil structure. The value of D for the condition of added FAA is about 0.6 times the value of D for zero number of primary roots (soil alone).

Curve D in Figure 16 shows the increased oxygen diffusion coefficient of a soil core when rice (var. Caloro) roots penetrated the soil. The points are quite scattered but this may be due to the difficulty of determining the number of primary roots. This is because it is difficult to distinguish between the primary roots and the large number of lateral roots possessed by rice seedlings. The average increase, slope of the curve, for the oxygen diffusion coefficient for rice (curve D) was about one-fourth that of corn roots (curve C).

One measurement of the oxygen diffusion coefficient for the soil core with rice was made after the soil core had been kept flooded for 8 days with the water level at the surface of the 5-mm. glass beads. The water was allowed to drain through the soil before the experimental run was started.
The oxygen diffusion coefficient was then found to be zero as shown in Figure 16, by the point labeled "flooded". During the period of flooding the openings of the plexiglas cylinder below the soil were sealed so that air trapped in the space below the soil prevented the water above the soil from draining into this space. During this time the roots below the soil showed no apparent change.

To determine whether the observed enhancement of oxygen diffusion through soil in the presence of plant roots was a result of oxygen movement through the root itself or was due to an effect of the root on the air-filled soil porosity, two further experiments were conducted.

Figure 17 shows the experimental container used for these experiments. In the first experiment a layer of 500\(\mu\) glass beads was supported from below by a single layer of tissue paper on top of plastic screen. The thickness of the glass bead layer was 1.7 cm. The glass beads were saturated with water and were maintained in a saturated condition during the experiment by the daily addition of excess water. \(^{18}\)O-enriched air diffused downward through the soil core and through the glass beads into the unenriched air stream. Holes for single-inlet sample tubes in the plexiglas container below and above the glass beads permitted collection of air samples for determining the \(^{18}\)O-enriched oxygen concentration gradient across the glass bead layer. Figure 9 indicates the
Figure 17. Experimental container for the determination of steady state oxygen diffusion through water-saturated, 500 μ, glass beads penetrated by corn seedling roots
REMOVABLE COVER

OUTLET FOR $^{18}O$-ENRICHED AIR

HOLE FOR INSERTION OF AIR SAMPLE TUBE

TISSUE PAPER SUPPORTED BY PLASTIC SCREEN

OUTLET FOR UNENRICHED AIR

SCALE: 3/4

18 SEEDLINGS

INLET FOR $^{18}O$-ENRICHED AIR

SATURATED 500 $\mu$m GLASS BEADS

INLET FOR UNENRICHED AIR

70 MM
The roots of 18 corn seedlings, which had been planted in the soil above the beads, were permitted to grow through the glass bead layer. Measurements of the oxygen diffusion rate through the glass bead layer were made as additional roots continued to grow through the layer. The results of this experiment are shown in Figure 18, together with a redrawn curve (top curve of Figure 18) for that of curve C of Figure 16. One sees in Figure 18 that the oxygen diffusion coefficient for corn roots growing through the saturated glass beads is small compared with that for corn roots growing through soil (top curve). The initial measurement (with 17 roots through the glass bead layer) shows a definite increase in the oxygen diffusion coefficient. The subsequent measurements on the saturated glass beads were based upon mass $^{34}/$mass 32 increases near the lower limit of detection by the mass spectrometer.

In the second experiment the 500$\mu$ glass bead layer (see Figure 17) was replaced by a layer of soft wax 0.57 cm. thick. The wax layer was sealed to the wall of the plexiglas container and supported underneath by a single layer of cotton gauze. The gauze prevented the wax from sagging at the higher temperatures occurring in the indoor greenhouse. The wax was "light green rosebush wax" and was purchased from National Wax Company, Skokie, Illinois. This is a paraffin base wax
Figure 18. Diffusion coefficient of oxygen vs. number of growing roots for various media (including the roots growing through them): a) Webster silty clay loam soil, b) saturated 500μ glass beads, and c) rosebush wax
ROOTS IN SOIL GLASS BEADS A WAX

NUMBER OF PRIMARY ROOTS
formulated for use with plants. The absence of any apparent effect of this wax upon the physiology of plant tissues has been confirmed by Toy (1958). An initial measurement without plants showed that no measurable amount of oxygen diffused through the wax.

Eighteen corn seedlings were planted in the soil above the wax and the primary roots were allowed to grow through the wax layer below the soil. Measurements of the oxygen diffusion rate through the wax layer were made when the roots had begun to emerge from the bottom of the wax layer. The results are shown in Figure 18. All of the measurements showed a very small coefficient of diffusion although there is a slight trend toward increased diffusion as the number of roots increased. It is apparent that the corn roots did not enhance the rate of oxygen diffusion through wax to any degree comparable to the enhancement in soil. In addition, this experiment indicates that there was no large transport of oxygen through the root itself. At least, if such transport occurred, the oxygen was not released from the roots below the wax.

An examination of the intercellular air spaces of the corn roots was made to determine whether the criterion of McPherson (1939) could be applied to give further information about the oxygen supply of these roots. McPherson found, it is remembered, that the large (lysigenetic) air spaces in
corn roots were evidence of a scarcity of oxygen in the medium surrounding the roots and, presumably, within the roots.

Corn roots were collected from the soil above the wax, from within the wax, and from the container below the wax. These roots were embedded and sectioned according to standard paraffin techniques and stained with safranin and Mayer's hemalum (Sass, 1958). Photomicrographs of three of these root sections are shown in Figures 19, 20 and 21. Figure 19 is a cross-section of a corn root from the soil above the wax. Several large air spaces occur in the cortex, indicating, according to McPherson (1939), that the oxygen supply of this root was less than optimum. Figure 20 is a cross-section of a corn root from within the wax layer. The lysigenetic air spaces are large and occupy the major portion of the cortex, indicating a distinct shortage of oxygen. Figure 21 is a cross-section of a corn root in the air below the wax. This root had an optimum supply of oxygen from the air which continually flowed past it in the plexiglas cylinder. The large air spaces are few in number.

Evidently, diffusion of oxygen through the root longitudinally was not sufficient to prevent the formation of large air spaces in the root shown in Figure 20. This agrees with the oxygen diffusion coefficients of Figure 18 for the roots in wax. The slight increase, with increasing root number, of the oxygen diffusion in Figure 18 might have
Figure 19. Cross section of corn root growing in soil. There are several large (lysigenetic) air spaces present in the cortex.

Hemalum and safranin stains, 10µ, X 95

Figure 20. Cross section of corn root growing in wax. There is extensive development of large (lysigenetic) air spaces in the cortex.

Hemalum and safranin stains, 10µ, X 95

Figure 21. Cross section of corn root growing in air. There is a minimum of large (lysigenetic) air space development.

Hemalum and safranin stains, 10µ, X 95
resulted from the development of the large air spaces; for
the formation of these large air spaces would increase the
limited oxygen diffusion through the wax.

Experiment on Soil-Root Oxygen Supply

Preceding experiments have indicated that the oxygen
diffusion rate through the soil was increased when corn roots
were present but that the corn root tips were not releasing
oxygen into the air surrounding them. The question arises
as to whether the increased oxygen diffusion for the soil
core occurred in the immediate vicinity of the root or ex­
tended outward an appreciable distance from the root. An
experiment was devised to attempt to answer this question.

The experimental container, with soil and plants, is
shown in Figure 22. A plexiglas cylinder containing soil is
divided in half by a partition above the soil and extending
into the soil 2 cm. A space below the soil, as indicated in
the figure, is separated from the soil by a rosin-wax mem­
brane, approximately 1 mm. thick, through which plant roots
may penetrate and emerge into the sealed space. The mem­
brane was prepared by the method of Stone and Mulkey (1961).
In the completed apparatus, nine corn seedlings were planted,
in a layer of 5 mm. glass beads placed above the soil, on
one side of the partition. The roots from these seedlings
then grew throughout the soil core below the partition.
Figure 22. Experimental container for the determination of the mixing ratio, \((\text{O}^{18}\text{-enriched oxygen})/(\text{O}^{18}\text{-enriched oxygen} + \text{unenriched oxygen})\), as a function of the air-filled soil porosity.
REMovable cover

Hole for circulation of unenriched air

Hole for inserting air sample tube

Scale: 3/4

9 seedlings

Hole for circulation of O^{18}-enriched air

5 mm glass beads

Soil

83 mm

Rosin-wax membrane
Several of the roots penetrated the rosin-wax membrane and entered the space below. An opening in the plexiglas wall below the rosin-wax membrane permitted sampling of the air in this space with a single-inlet sample tube.

$^{18}O$-enriched air was passed through the half of the plexiglas cylinder containing the corn seedlings, and unenriched air was passed through the other half of the plexiglas cylinder. If oxygen diffuses through both halves of the soil core at equal rates, then the ratio in which the $^{18}O$-enriched air and the unenriched air are mixed in the soil just above the rosin-wax membrane should be 1:1. That is, the ratio, ($^{18}O$-enriched oxygen)/(total oxygen), which will subsequently be referred to as the mixing ratio, should be 0.5. If the effect of the roots in increasing the oxygen supply is extended throughout the soil, then the mixing ratio will be approximately 0.5. On the other hand, if the effect is limited to the volume occupied primarily by the roots, then more oxygen-18, from the half of the container in which the corn seedlings were planted, would be present in the soil and roots above the rosin-wax membrane; and the mixing ratio will be greater than 0.5.

If diffusion of oxygen through the rosin-wax membrane can occur only through the roots penetrating the membrane, then the oxygen in the space below the rosin-wax membrane should have the same peak ratio, mass 34/mass 32, as the
oxygen within the interior of the root, after sufficient time for equilibration has elapsed. This should be true even though oxygen exchange between the roots and the surrounding air is quite limited.

The mixing ratio and total oxygen concentration below the rosin-wax membrane are shown as a function of the air-filled soil porosity in Figure 23. The initial measurement was made at an air-filled porosity of 0.17 and subsequent measurements were made after applications of water to the soil to decrease the air-filled porosity. The oxygen percentage is approximately linearly related (see solid points) to the air-filled porosity, decreasing from 20% at a porosity of 0.17 to 2% at a porosity of 0.07. As for the open points, it is noted that the fitting of a curve is not pertinent. What is pertinent is the fact that most of the points lie below an ordinate (mixing ratio) value of 0.5. The mean mixing ratio for these points is 0.39. As was indicated, this value of less than 0.50 means that less than one half of the oxygen in the sealed chamber is $^{18}\text{O}$-enriched oxygen and that, therefore, the half of the soil core where most of the roots were located was conducting less oxygen than the other half. In other words, the roots appear to have decreased diffusion in the half of the soil core containing plants, possibly by increased consumption of oxygen. There is an alternate conclusion. The two halves of the soil core may have had
Figure 23. Mixing ratio, \((^{18}\text{O}_{2}\)-enriched oxygen)/(\((^{18}\text{O}_{2}\)-enriched oxygen + unenriched oxygen), and percent oxygen below rosin-wax membrane as a function of the air-filled soil porosity. The straight line applies only to the solid points.
different coefficients of oxygen diffusion initially. Further experiments of this type are needed to clarify the results. The value of the mixing ratio for the soil core without plants should be determined initially. In addition, this experiment should be repeated without the rosin-wax membrane below the soil in order to determine its effect on the results.

Experiment on Photosynthetic Oxygen in a Soil-Plant System

This experiment was devised to show whether there was any preferential movement of photosynthetic oxygen into rice roots in flooded soil. This objective was not attained but some side results that are of interest were obtained. The procedure and setup were as follows. After the oxygen diffusion measurements through the soil containing rice roots (curve D in Figure 13) were completed, the soil was again flooded to the surface of the 5-mm. glass beads. The inlet and outlet for unenriched air below the soil were stoppered and sealed with wax (see Figure 8). $^{18}O$-enriched air was passed above the soil as before. With this arrangement there was only two possible sources of any oxygen appearing in the space below the soil, after the oxygen originally present had been consumed. One possible source was the $^{18}O$-enriched air above the soil and the other was photosynthetic oxygen from the plants. Since photosynthetic oxygen arises from metabolic water within the plant tissue (Ruben et al., 1941),
it will have the same oxygen-18 concentration as the water, that is, essentially the same as atmospheric oxygen. As was stated in the review of literature, the metabolic and other water in the system will not exchange with the $^{18}_0$-enriched oxygen around the plant tops. Also, the water which is formed from the $^{18}_0$-enriched oxygen by respiration is negligible compared to the total quantity of water present. Therefore, the fraction of photosynthetic oxygen in the oxygen supply to the roots and soil could be evaluated from measurements of the peak ratio, mass $34/32$, in the air below the soil.

Before starting this experiment, the space below the soil was flushed with nitrogen gas. During the course of the experiment the sample tube used for collecting an air sample from the space below the soil was also flushed with nitrogen gas before insertion into the hole in the plexiglas cylinder (see Figure 8).

The measurement for the first day of the experiment showed that the total oxygen concentration below the soil was only $3.4\%$. The peak ratio, mass $34/32$, was the same as that of unenriched air. However, it cannot be concluded that the total oxygen concentration below the soil was photosynthetic oxygen; for this small quantity of oxygen may have been a residual amount which diffused out of the soil after the space below the soil was flushed with nitrogen gas. Measurements on four subsequent days bore out this possibility.
They showed that only a trace of oxygen was present below the soil. Consequently, it was not possible to draw any conclusion about the role of photosynthesis in supplying oxygen to the rice roots.

The rice roots below the soil appeared to be in good condition throughout and after the experiment. It appears that these roots either had access to a source of oxygen outside the soil, were capable of subsisting on a very low concentration of oxygen, or were obtaining oxygen by nitrate reduction.

A scan of the mass spectrum of the air below the soil was made for the last two days of the experiment, 6/13/61 and 6/14/61 (see Figure 24). Studying Figure 24, one sees that the mass 32 peak, which is an index of the total oxygen concentration, was very small. In fact, when one compares the mass 32 peak with the peak, in the figure, for argon (mass 40), it is seen that, for both days, the mass 40 peak height is at least 4.5 times the mass 32 peak height. Therefore, the oxygen (mass 32) concentration must have been less than 0.2%, since argon constitutes 0.94% by volume of ordinary air.

In addition, if these two mass spectra are compared with the mass spectrum of ordinary air (unenriched air of Figure 2), it is seen that the argon peak (mass 40) is greater than the $^{14}_N^{15}_N$ peak (mass 29) for ordinary air, whereas the
Figure 24. Mass spectra of air below flooded Webster silty clay loam soil containing rice seedling roots, for the two days, 6/13/61 and 6/14/61. The peaks with numbers 14, 14.5, 15, 28, 29 and 30 represent nitrogen isotopes. Numbers 16 and 32 represent oxygen-16. Number 18, water. Numbers 20 and 40, argon. Numbers 22 and 44, carbon dioxide.
reverse occurs in the two spectra of Figure 24. This indicates that nitrogen gas was being evolved from the soil. This, in turn, indicates that considerable denitrification was occurring in the soil. Oxides of nitrogen do not appear in the mass spectra because they are removed, if present, by the liquid nitrogen cold trap.

The mass spectrum for 6/14/61 (Figure 24) contains a prominent mass 44 peak and a small half-mass peak at mass 22. This is the result of carbon dioxide in the air sample which passed through the liquid nitrogen cold trap without freezing. This fact indicates that a large quantity of carbon dioxide was present, for all of the carbon dioxide would freeze out in the trap if only a small quantity were present in the air sample when analyzed.
DISCUSSION

The preceding sections have presented successful procedures and apparatus, and some applications, demonstrating the feasibility of using the stable isotope, oxygen-18, as a tracer of oxygen diffusion in soils. The experiments presented were limited in scale and complexity, but there is no apparent reason why extension of the methods to larger and more complicated experiments should not be possible. Enlargement of the electrolysis train to produce $^{18}$O-enriched air at a greater rate could be accomplished with the modification, proposed earlier, of the platinum electrode leads to permit use of KOH as an electrolyte and, consequently, larger electrolyzing currents. The provision of a means for continuous mixing of the electrolytic solution would allow experimental runs to continue for several days, during which changes in the aeration conditions of the system could be followed. Because the cost of $^{18}$O-enriched deuterium oxide is small (25 cents/g.), the use of oxygen-18 as a tracer in small field experiments may be practicable.

The experiments which have been described were intended to explore some applications and possible results of using oxygen-18 as a tracer. Effective oxygen diffusion coefficients for soils were measured with a standard deviation estimated to be $8 \times 10^{-6}$ cm.$^2$/sec. This standard deviation could be reduced in future work by further refinement of the apparatus.
and techniques, if higher precision is needed, for example, in measuring oxygen diffusion in plant tissue.

The curves which were obtained relating the oxygen diffusion coefficient to the air-filled soil porosity showed little scatter (see Figures 11, 12 and 13). This lack of variation may have resulted from the elimination of errors due to respiration at the soil surface and from the precision of the mass spectrometer measurements.

An enhancement of oxygen diffusion through the soil was observed at low air-filled porosities whenever corn or rice roots were present in the soil (see Figures 15 and 16). This is the opposite of what one would expect in view of the increased respiration rate when roots are present. The oxygen diffusion coefficient with roots present did not show a close relationship to the air-filled soil porosity as it did when roots were absent (see Figure 15).

From results shown in Figure 18 it is possible to calculate the coefficient of oxygen diffusion in the root itself, assuming that all of the oxygen moves in the root. For the corn roots in soil (Figure 18), the initial slope of the curve provides a value of 0.236 cm$^2$/sec. if the root diameter is taken as measured, 0.81 mm. This value is greater than the coefficient of oxygen diffusion in air (0.212 cm$^2$/sec.). For the corn roots in wax (Figure 18), the calculated value of the coefficient of oxygen diffusion
is $0.178 \times 10^{-2} \text{ cm.}^2/\text{sec}$. This value is much larger than the value of $11 \times 10^{-6} \text{ cm.}^2/\text{sec}$ which Berry and Norris (1949) determined for excised onion roots. The large (lysigenetic) air spaces formed in the corn roots (see Figure 20) in wax may account for the higher rate of oxygen diffusion observed in these roots.

Further experiments were performed to determine whether the increased oxygen diffusion in the presence of roots takes place in the soil or within the roots. The results, listed as items 1 to 4 below, support in a general way the hypothesis that nearly all of the observed oxygen diffusion occurred in the soil:

1. The observed oxygen diffusion coefficient was very small when the roots grew through saturated 500$\mu$ glass beads or through a soft wax (see Figure 18). However, one value for the saturated glass beads was about 4 times the estimated standard deviation for this type of measurement. This larger value may have resulted from the glass beads becoming less than saturated during the experimental run, although this was not apparent from the appearance of the beads.

2. Large (lysigenetic) air spaces were formed in the corn roots growing in wax which was 0.57 cm. thick (Figure 19). This indicates, according to McPherson (1939), that there was a shortage of oxygen in these roots, that is, oxygen diffusion through these roots was inadequate prior to forma-
tion of the large air spaces.

3. The increased oxygen diffusion through soil with rice roots present was eliminated by flooding the soil (see Figure 16). In addition, no measurable amount of oxygen was released from rice roots growing in a closed air space below flooded soil (see Figure 14).

4. In the experiment on soil-root oxygen supply, the mixing ratio was always less than, or equal to, 0.5 (see Figure 23), indicating that as much oxygen was supplied from the half of the container without plants as from the half with plants. That is, the oxygen appeared to enter the roots below the rosin-wax membrane by a pathway which included, at least in part, the soil.

The phenomena associated with the increased oxygen diffusion rate in the presence of plant roots in soil were not indicated by the results of these experiments. The cause may be simply a redistribution of the soil moisture resulting in an arrangement of the air-filled porosity more conducive to diffusion. Further investigation of this subject is necessary. The other experiments which have been described should also receive further investigation. More plant species and types of soil should be tested.
SUMMARY AND CONCLUSIONS

An electrolysis train (Figure 1) was devised and developed for producing two equal air streams, one of which was enriched with $^{16}O^{18}$. The two streams were used to sweep out different parts of an experimental soil container (Figures 8, 9, 10, 17 and 22) in order to set up a steady state condition for the $^{18}$-enriched oxygen diffusing in the soil. Air samples taken from the experimental container after the steady state had been established were analyzed to determine the concentration of $^{18}$-enriched air present. The movement of the $^{18}$-enriched oxygen in the soil could be determined from these concentrations.

The effective coefficient of oxygen diffusion $D$ as a function of the air-filled soil porosity was determined for three soil cores (Figures 11, 12 and 13). Comparison of these results with those of previous workers showed good agreement (Figure 14). The standard deviation of the measurements of $D$ was estimated to be $8 \times 10^{-6}$ cm$^2$/sec.

The value of $D$ was increased when corn or rice roots grew through the soil cores, but not when corn roots grew through soft wax or water-saturated 500μ glass beads (with the exception of one measurement for the glass beads) (Figure 18). A characteristic curve was found relating $D$ and the number of roots growing through very wet soil. The curve has a maximum followed by a sharp decrease as the number of
roots increases (Figure 16). The evidence (presented in the discussion) supports the hypothesis that the increased value of D with corn roots present represents, almost entirely, oxygen moving in the soil rather than in the roots.

An experiment to determine the relative effectiveness of corn roots and soil in supplying oxygen to the roots indicated that most of the oxygen reached the roots by a pathway through the soil (Figure 23); since oxygen diffused at approximately equal rates into soil with, and without, roots at the surface and contributed to the oxygen supply of roots at a lower level.

An experiment to determine whether photosynthetic oxygen was supplied to rice roots in flooded soil was inconclusive because only a trace of oxygen could be detected below the soil.

Conclusions for the experiments may be set down as follows.

1. Oxygen-18 may be used as a tracer of oxygen diffusing in soils and soil-plant systems (Figures 11, 12, 13 and 14) under steady state conditions.

2. Apparatus may be constructed for producing a continuous stream of $^{18}$O-enriched air by electrolysis of $^{18}$O-enriched $D_2O$ or $H_2O$ and used for studying diffusion processes in soils and soil-plant systems.

3. The coefficient of oxygen diffusion in Webster silty
clay loam soil was increased when corn or rice roots grew through the soil (see Figures 15 and 16).

4. Evidence was obtained (see discussion section) that the observed increase of oxygen diffusion through soil in the presence of plant roots took place almost entirely in the soil, rather than in the root.


ACKNOWLEDGMENTS

The author wishes to acknowledge the guidance, suggestions and encouragement of Dr. Don Kirkham, Professor of Soils and Physics at the Iowa State University of Science and Technology.

The author further wishes to acknowledge the joint financial assistance of the Iowa Agricultural Experiment Station and the Institute for Atomic Research for the support of this work.

In addition, the author wishes to thank his wife, Jo Ann, for her help and encouragement during the period of graduate study.