0038-075X/86/1426-0352$02.00/0
SOIL SCIENCE

December 1986
Vol. 142, No. 6
Printed in U.S.A.

DYNAMICS OF PARTIAL ANAEROBIOSIS, DENITRIFICATION, AND WATER IN A SOIL AGGREGATE: EXPERIMENTAL

P. A. LEFFELAAR[1]

A respirometer system was developed to study the dynamics of partial anaerobiosis and denitrification in unsaturated soil. The system enables one to measure simultaneously the distribution of water, oxygen, nitrate, nitrite, ammonium, and pH as a function of space and time in an unsaturated, artificially made, homogeneous, cylindrical aggregate and the changes in atmospheric composition as a function of time in the chamber that contains the aggregate. Except for water transport, these processes are caused by microbial activity, because roots are not present in the aggregate.

The respirometer system was especially designed to generate coherent data sets to evaluate a simulation model that calculates the development of denitrification products as a function of environmental conditions. Nondestructive measurements during an experiment involve gamma-ray attenuation, gas chromatography, and polarography. Destructive measurements are executed at the end of an experiment in the form of chemical analyses of soil.

The reported experiment shows that hysteresis in the soil water characteristic strongly affects the water distribution in the aggregate. As a result, the oxygen supply to the interior of the aggregate is decreased to such an extent that anaerobiosis is maintained there after the oxygen is consumed. The respiratory quotient and the release of denitrification products are underestimated in the partially wet soil because of the high solubilities of carbon dioxide and nitrous oxide in soil moisture. Large amounts of nitrite have been found. Therefore, assessment of denitrification through the measurement of nitrate alone will overestimate nitrogen losses, while the measurement of nitrous oxide and molecular nitrogen alone will given an underestimation. The consumption rate of oxygen and the production rates of carbon dioxide, nitrous oxide, and molecular nitrogen compare well with field data. This is the result of the pretreatment of the soil, which aimed at avoiding the flush of microbial activity upon wetting. The results support the thesis that denitrification will occur in soil when at a certain place and time oxygen is absent and bacteria capable of denitrification, water, nitrate, and decomposable organic compounds are present.

The respirometer system yields valuable data to evaluate the simulation model. However, full account of the interrelationships among the generated data can be achieved only by the same simulation model, because the measured variables reflect the integrated effect of biological activity and transport processes.

The objective of this paper is to describe the respirometer system and its measuring devices and to report some of the measurements.

The release of nitrous oxide and molecular nitrogen by biological denitrification occurs when bacteria capable of denitrification colonize a location where oxygen is absent and water, nitrate, and decomposable organic compounds are present (Delwiche 1981; Ingraham 1981). In aggregated unsaturated soils anaerobiosis, and hence denitrification, is mainly confined to the aggregates (Currie 1961; Greenwood 1961). In principle, therefore, denitrification losses from aggregated field soils can be predicted when denitrification losses from individual aggregates and their size distribution are known (Smith 1977 and 1980). Denitrification from a single aggregate can be predicted successfully only when the spatial distributions of denitrifiers, oxygen, water, nitrate, and decomposable organic compounds can be measured or calculated as a function of time and when these distributions are subsequently combined so that zones of denitrification show up.

Figure 1 depicts some schematic oxygen and water distributions as expected in field aggregates under the assumption of a homogeneous distribution of bacteria and organic compounds

[1] Dept. of Theoretical Production Ecology, Bornsesteeg 65, 6708 PD Wageningen, The Netherlands.

Received for publication 6 May 1986; revised 11 Aug. 1986.

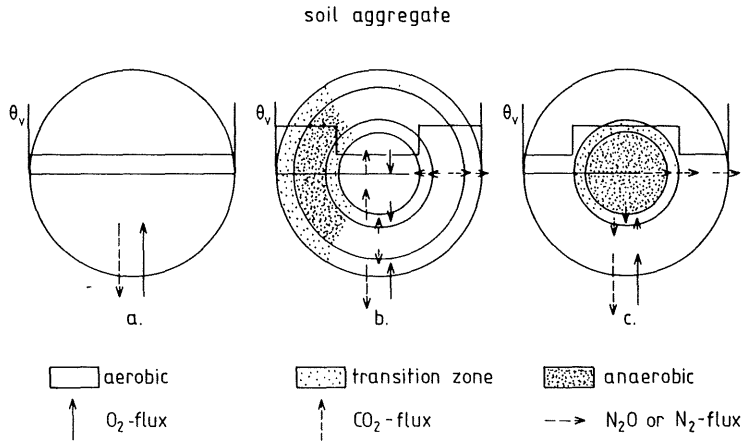

FIG. 1. Schematic of water and oxygen distributions in soil aggregates. a, in a dry period; b, just after a rain shower; c, some time after rainfall. Lengths of arrows indicate relative magnitudes of molar source or sink terms.

and a negligible nitrate production due to nitrification. When oxygen consumption rate does not exceed oxygen supply rate, no anoxic conditions develop, and equimolar respiration occurs as indicated by arrows (Fig. 1a). Just after rainfall, mainly the outer shell of an aggregate will be wetted (Leffelaar 1979). In the outer shell oxygen diffusion rate is then seriously impeded, and when oxygen consumption rate exceeds oxygen supply rate, anoxic conditions occur there (Fig. 1b). When nitrate from fertilizer has been absorbed with the rainwater, denitrification occurs in the wetted shell. In the center of the aggregate equimolar respiration continues to take place until the oxygen from the enclosed air has been consumed. Then most of the aggregate volume is anaerobic, but denitrification does not necessarily increase, for the nitrate is mainly concentrated in the wetted shell. The arrows in Fig. 1b indicate a net gas production caused by denitrification. Subsequent redistribution of water may result in a decrease of the anaerobic aggregate volume, and hence of denitrification, when the water content in the wetted shell becomes low enough to get continuous gas-filled pores that permit rapid oxygen diffusion into the aggregate. The distribution of oxygen as depicted in Fig. 1c will be found in initially water-saturated aggregates that are drying. Upon further drying their oxygen distributions will adjust to that of Fig. 1a.

These complicated dynamic interactions between biological and physical processes determining denitrification are studied in a most comprehensive way through the development of an explanatory simulation model that describes microbial activity; movement of gases, water, and nitrate; and decomposition of organic compounds in an individual aggregate. It appeared that coherent data sets to evaluate such a comprehensive model did not exist. It is the objective of this paper to describe a new type of respirometer system designed especially to generate such coherent data sets and to report some of the measurements.

MATERIALS AND METHODS

The experimental soil aggregate on which the measurements of the spatial distribution of oxygen, water, nitrate, and decomposable organic compounds as a function of time are performed is a short cylinder of homogeneously packed soil material in which transport processes are radial. This geometry is a model representation of a soil aggregate from which the upper and lower sides are removed and originates directly from Fig. 1. For the same geometry a simulation model is developed that can thus be evaluated. The evaluated model may then be converted to a spherical geometry and be used in a field model describing the spatial arrangement of aggregates and the interaggregate transport processes. Thus, the interaction between microbial activity and the movement of gases, nitrate, and water and with this denitrification in real situations can be simulated. A scheme of the experimental installation is given in Fig. 2.

Soil

A sandy loam soil from Lelystad was taken from the surface 25-cm layer and stored under field-moist conditions. Some characteristics are: pH (measured in 4 g of soil suspended in 10 ml of liquid) in H_2O and KCl: 7.8 and 7.4, respec-

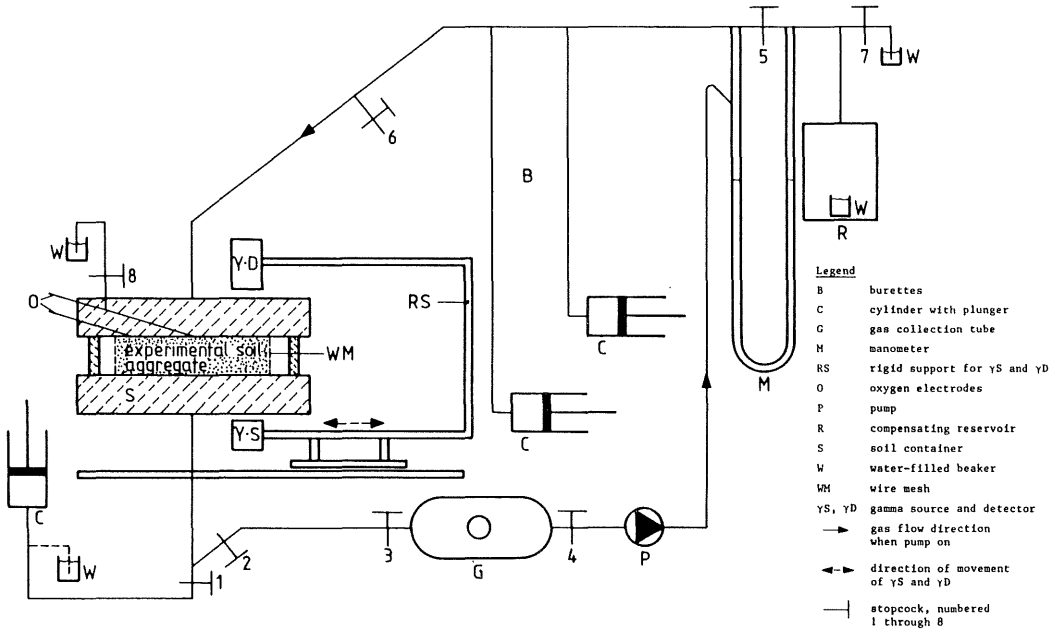

FIG. 2. Diagram of respirometer system showing soil container, gas collection circuit (stopcocks 1 through 7 and attached devices), oxygen electrodes, and gamma-ray attenuation apparatus with rigid support. Spatial arrangement of oxygen electrodes is correctly shown in Fig. 3.

tively; $CaCO_3$ (Scheibler's method described by Allison and Moodie 1965): 6%; organic carbon (Mebius 1960): 1.2%; total nitrogen (Novozamsky et al. 1984): 0.09%; CEC-$BaCl_2$ (Bascomb 1964): 10.1 cmol(+) per kg of soil and soil texture (pipette method described by Day 1965) <2 μm, <20 μm, and <50 μm: 13, 27, and 32%, respectively. The soil is air-dried, pulverized to pass a 0.5-mm sieve, directly remoistened to about 0.1 to 0.15 g g^{-1}, and left in a closed container for at least 6 wk to let the metabolism of microorganisms recover (Birch 1958, 1959; Fillery 1983). Remoistening is attained by adding an appropriate amount of ice scrapings to soil and mixing in a mortar with a pestle.

Measuring system

The measuring system (Fig. 2) was mounted in a constant-temperature room (22.7 ± 1.5°C) and is composed of the following subsystems

1. container (S), which holds the experimental soil aggregate and the oxygen electrodes
2. a closed gas collection circuit (stopcocks 1 through 7 and attached devices), which serves three purposes
   —it contains enough oxygen to meet the respiratory demand of the microorganisms in the soil aggregate during one experiment
   —it functions as volumeter to determine net changes in the total volume of gas resulting from respiration and denitrification
   —it is used to withdraw gas samples to assess gaseous composition by gas chromatography
3. two polarographic Clark-type oxygen electrodes to measure oxygen pressure at two locations in the experimental soil aggregate
4. a gamma-ray attenuation apparatus to measure the radial distribution of soil water content

Technical details, comments on procedures, and calculations to obtain data are given below for each subsystem. Table 1 summarizes the course of an experiment.

Soil container

Technical description

Figure 3 depicts the Perspex soil container in which the cylindrical soil aggregate is radially

TABLE 1

Course of experiment

Initial

Preparation

1. Weigh (W1) empty soil container (*S*); pack experimental soil aggregate in container and weigh (W2) again. Connect container to gas collection circuit, of which stopcocks 1 through 7 (glass) are greased by vacuum grease (Dow Corning). (W2 − W1) gives amount of initially moist soil.
2. Leave soil aggregate alone for at least 1 wk to let metabolism of microorganisms recover; closed stopcocks: 1, 5, 6, 7, 8. Stopcocks not mentioned are open.
3. Replace air by a mixture of neon-oxygen (about 0.2 ml O_2 ml^{-1}) (Matheson Gas Products, Oevel, Belgium) during this week by periodic flushing through stopcock 6 while pump is switched on; closed stopcock: 8, water-sealed stopcocks: 1, 7.
4. Record gamma-ray attenuation reference scans for moisture calculations and for assessing homogeneity of packing. Record at least five scans with a measurement every third millimeter.
5. Test (qualitatively) whether soil respiration occurs through gas analysis for carbon dioxide. If no respiration takes place, experiment may be stopped.
6. Determine initial moisture content and NO_3^-, NO_2^-, NH_4^+, and pH on separate amount of soil.

Start

7. Calibrate polarographic oxygen electrodes by bathing them in gas mixtures (Matheson Gas Products) containing known volume fractions of oxygen in nitrogen (about 0.0, 0.05, 0.1, and 0.2 ml ml^{-1}) and noting the mV signal.
8. Adjust burettes to half of their maximum volume.
9. Install oxygen electrodes with pump switched on, and flush system with Ne-O_2 mixture through stopcock 6 to prevent air entry; closed stopcock: 8, water-sealed stopcocks: 1, 7. Leak-proof connections are attained by piercing the electrodes through greased rubber stoppers fitting into the borings of the top cover of the soil container.
10. (Partially) wet experimental soil aggregate via cylinder with plunger near stopcock 1 with solution either with or without nitrate and/or glucose added; closed stopcock: 2, water-sealed stopcock: 7. Enclosed air in soil is allowed to escape via tube containing central oxygen electrode and water-sealed stopcock 8.
11. When solution has penetrated to desired depth into soil aggregate, excess water is removed by withdrawing plunger of cylinder near stopcock 1; closed stopcocks: 2, 8, water-sealed stopcock: 7.
12. Continue to flush system with Ne-O_2 mixture for about 1 min; closed stopcocks: 1, 8, water-sealed stopcock: 7.
13. Time is zero when stopcocks are closed in the sequence 6, 7, 5, and pump is switched off.

Time elapsed from step 8 through step 13 is about 8 min.

Dynamic

Measurements as a function of time and place

14. Oxygen pressure by polarography.
15. Moisture by gamma-ray attenuation.

Measurements as a function of time

16. Gas analysis for Ne, CO_2, N_2O, O_2, and N_2 by gas chromatography. Before moment of gas sampling: adjust system pressure via burettes and manometer and switch on pump for 10 min. At moment of sampling: switch off pump, readjust system pressure if necessary, and close stopcocks 3 and 4. Analyze gas sample from gas collection tube. If gas analysis seems not trustworthy, a new gas sample can be taken. Open stopcocks 3 and 4. Calibration chromatograms are recorded regularly.
17. Read volume of burettes.
18. Read temperature.

Steps 14 through 18 can be taken as simultaneous, as the rate of measuring is much faster than the rate of change of the state variables measured.

Gamma-ray attenuation measurements take 20 min and are started 10 min before the moment that the other readings are performed.

Terminal

Preparation

19. Take out oxygen electrodes and weigh container with soil (W3). (W3 − W2) gives amount of water added during wetting.
20. Calibrate oxygen electrodes.

Measurements as a function of place

21. Partition soil aggregate into five concentric layers of 1-cm thickness each.
22. Determine gravimetric water content and NO_3^-, NO_2^-, NH_4^+, and pH in each layer.

Steps 19 through 21 take less than 1 h. Soil samples for chemical analyses are put into extraction solutions within 2 to 3 h to prevent further microbial conversions of nitrogenous and carbonaceous materials. Gravimetric water contents are used to express chemical data on an over-dry basis.

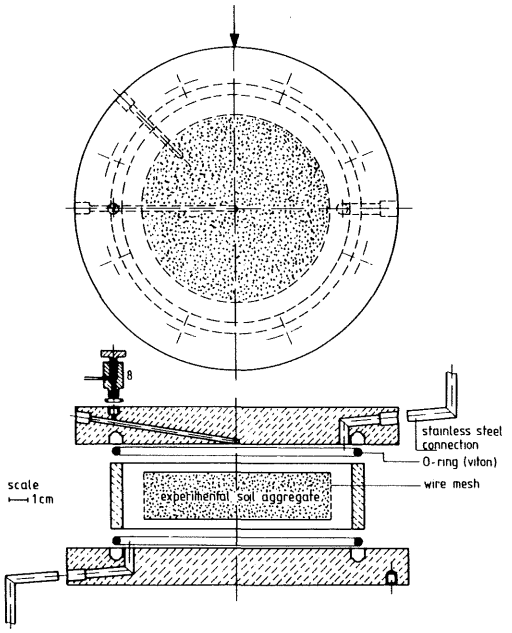

FIG. 3. Top cover of soil container seen from above and expanded transect through container center. Drawn to scale. Arrow indicates direction of movement of gamma-ray source and detector.

supported by a wire mesh of 72.7% porosity. The upper and lower sides of the aggregate are covered by two disks that are kept apart by a cylinder of 12-cm internal diameter. The aggregate has a height and diameter of about 2.5 and 10 cm, respectively, to provide enough soil for chemical analyses on concentric rings of 1-cm thickness each. Nuts to tighten the disks with the soil in between have been sunk into the Perspex to minimize the distance between the source and detector of the gamma-ray attenuation apparatus. In the upper disk two 2.1-mm-diameter holes were drilled from the circumference to the center of the soil container and to a place 4 cm from the center, respectively. Each hole may accommodate a 2-mm-diameter Clark-type oxygen electrode and slants toward the inner part of the soil container to keep the head space between the electrode tip and the soil surface as small as possible. A brass stopcock (no. 8) with a small dead volume is connected with the central electrode hole to let enclosed gases escape from the soil aggregate when it is partially wetted at the start of an experiment. Each Perspex disk has one 5-mm-diameter hole that connects the gaseous atmosphere around the aggregate with the gas collection circuit via a stainless steel tube glued into the Perspex. The holes are placed opposite one another with respect to the center of the container to force the gases in the system around the soil aggregate to mix thoroughly before gas samples are taken. In the bottom disk two holes (one visible in transect in Fig. 3) are drilled from below to fasten the soil container onto a steady support.

Comment on procedures (Table 1, item 1)

The experimental soil aggregate is prepared in an auxiliary cylinder, which is placed into the previously weighed (W1) soil container. The wire mesh for radial support of the aggregate fits loosely into this auxiliary cylinder, which has a height and internal diameter of 2.5 and 9.8 cm, respectively. A quantity of sieved moist soil, sufficient for an aggregate with a dry bulk density of 1.4 g cm^{-3}, is weighed. A small amount of this soil is then added to the auxiliary cylinder and tapped as homogeneously as possible, before the next amount of soil is added. This process is repeated until the appropriate height has been reached. Then the auxiliary cylinder is pulled up, excess soil is removed, and the container is assembled and weighed (W2).

Gas collection and analysis

Technical description

The gas collection circuit contains the following parts (Fig. 2)

1. a volumeter composed of a manometer (glass, internal diameter 0.4 cm), two calibrated burettes (glass, 50- and 100-ml capacity) of which the water levels can be adjusted by cylinder-plunger arrangements (Perspex), and a compensation reservoir (Perspex, 2500-ml capacity).

   The initial volume of the gas circuit (about 550 ml, including the space around the soil aggregate) is determined by the sum of the differences in weight of each part when filled with water and when dry, respectively.

   Pressure differences between the gas circuit and the compensation reservoir are eliminated by adjusting the water levels in the burettes. The change in burette volume is a measure for the net change in gas volume due to respiration and denitrification in the soil aggregate. The compensation reservoir is added to avoid the

influence of barometric pressure changes on the burette volume readings during an experiment. To maintain similar water vapor pressures at both sides of the manometer, a beaker filled with water is placed in the compensation reservoir (Scholander 1942).

2. a gas collection unit composed of a small pump (aluminum pump head, viton diaphragm, and valves, modified to reduce pressure buildup to 5 cm H_2O, flow rate 170 ml min^{-1}; Charles Austen Pumps Ltd., Surrey, England) to homogenize the gases evolved in the gas circuit and a gas collection tube (glass, 75-ml capacity, Subaseal septum), in which the mixture is trapped by closing stopcocks 3 and 4 at the moment that gas composition is to be determined.

   A preliminary experiment showed that gases are well mixed after 10 min of pumping. Gas samples are withdrawn from *G* through the septum by a 0.1-ml Hamilton 1710 RN gas syringe (Hamilton Co., Reno, Nevada, U.S.A.), which is previously flushed with carrier gas from the gas chromatograph to remove traces of air.

The different parts of the gas collection circuit are connected by glass tubes with internal diameters between 0.4 and 0.8 cm. Transitions between different parts are bridged by short pieces of gas-tight rubber tubes (viton and butylene tube), which are additionally tightened by Unex hose clips (Uniclip Ltd., Surrey, England).

Gas samples are injected in a Packard-Becker 427 gas chromatograph, which contains a Porapak (stainless steel, 400 cm long and 2-mm internal diameter, packed with 80–100 mesh Porapak Q; Supelco, Inc., Bellefonte, U.S.A.) and a Mol Sieve (SS, 400 cm × 2 mm, packed with 60–80 mesh Mol Sieve 5A; Supelco) column in a series/bypass configuration with restrictor (McNair and Bonelli 1969; Thompson 1977). The system is equipped with a thermal conductivity detector (TCD) with constant filament temperature (Packard Becker, Type 903 with Type 303 detector controller). Operating temperatures of injection port, column oven, valve, detector block, and filament are 70, 50, 70, 70, and 200°C, respectively. Hydrogen carrier gas (Matheson Gas Products, 99.999% purity) with a flow velocity of 20 ml min^{-1} is routed to the columns via a drying tube (15 cm × 10 mm) packed with granular Mol Sieve 5A (8–12 mesh; Supelco). A fast recovery of the base line after switching of the valve, is obtained by pressure control.

A gas analysis takes about 7 min, peaks are completely separated, and the minimal detectable volume fraction equals about 0.0003 ml ml^{-1} for CO_2 from air.

Calibration of the system is performed by analysis of a gas mixture (L'air Liquide Belge, Schelle, Belgium) containing known volume fractions of Ne, CO_2, N_2O, O_2, and N_2 (about 0.75, 0.05, 0.05, 0.1, and 0.05 ml ml^{-1}, respectively; relative accuracy 3%).

Comment on procedures (Table 1, item 3)

Replacement of air-N_2 by a gas of the helium group serves to overcome the experimental problem to determine small amounts of N_2 from denitrification in an atmosphere in which this gas is the major constituent. A gas of the helium group does not significantly affect denitrification (Blackmer and Bremner 1977) and, in addition, is used to indicate leaks in the gas collection circuit during an experiment by following its time course. Neon gas has been chosen because of its easy separation from oxygen and molecular nitrogen at temperatures slightly above room temperature.

Calculations

Gas percentages of the sample are calculated using peak areas and calibration chromatograms before and afer sampling. Gas percentages are converted to milliliters of gas using the volume of the gas collection circuit as adjusted by the volumeter. These state variables are further used to calculate production (CO_2, N_2O, N_2) and consumption (O_2) rates by numerical differentiation.

Oxygen pressure

Technical description

Oxygen pressure is measured in the center and 4 cm from the center of the experimental soil aggregate by means of Clark-type oxygen electrodes. The electrodes were purchased from the Department of Physiology, Faculty of Medicine, University of Nijmegen, The Netherlands, which also supplied the amplifier scheme. Electrode construction, operation, and characteris-

tics have been described by Kimmich and Kreuzer (1969) and Kreuzer et al. (1980). General schemes for amplification of oxygen electrode currents have been published by, among others, Prazma et al. (1978) and Fatt (1976).

The electrodes were selected for their small oxygen consumption, compared with that of soil. Typical values for the electrode and soil are 7.8 10^{-13} mol s^{-1} (using Faraday's law, Gleichmann and Lübbers 1960, and a current of 300 nA as measured at an oxygen pressure of 20 kPa with a 12-μm Teflon membrane mounted) and 9.6 10^{-6} mol m^{-3} of soil s^{-1} (assuming that 5 L O_2 m^{-2} d^{-1}, Grable 1966, is uniformly consumed over a depth of 0.25 m)[2]. Further criteria to select the electrodes were stability (usually less than 10% variation in sensitivity over 1 to 7 d of use), linearity (correlation coefficients of calibration lines before and after experiments up to 7 d were always significant at the 1% level), and smallness (2-mm diameter).

Electrode currents are preamplified and converted to voltages to yield a signal of 1 mV per nA. This signal is further processed by an electrical circuit that is used to select the appropriate polarization voltage of the electrode (adjustable between 0 and −1.56 V), to adjust its sensitivity (1 to 22 times) and its zero current (up to 1000 nA), and to suppress the signal as a whole (0 to −2 V) to record small changes in signal at high oxygen pressures. The electrode signal is read from a digital voltmeter and is continuously recorded on a strip chart recorder to note any sudden changes in signal.

[2] Comparison of these two numbers with different dimensions was done by calculating the time course of oxygen pressure in an air-filled chamber of 0.6-cm diameter and 0.01-cm length. The chamber was sealed at one side by an electrode and at the other side by the end of a soil pillar of similar diameter and a length of 2.5 cm. At time zero, oxygen pressure (pO_2) was 2 10^4 Pa, and no oxygen was supplied thereafter. Oxygen consumption of the electrode (O_{2el}, mol) was calculated by integrating $dO_{2el}/dt = (a\ pO_2)/(nF)$, where t is time (s), a is electrode sensitivity (A Pa^{-1}), n is number of moles of electrons involved in the reduction of one mole of oxygen (4), and F is the charge of one mole of electrons (Faraday's constant: 96494 A s mol^{-1} electrons). Oxygen consumption rate of soil was taken as mentioned above. The effective diffusion coefficient in soil (D) was calculated from $D = D_0\ (\epsilon\text{-}\theta_v)^{(4/3)}$ (Millington 1959), where D_0 is oxygen diffusion coefficient in free air, ϵ is soil porosity, and θ_v volumetric water content. Values for these parameters were 0.1987 10^{-4} m^2 s^{-1}, 0.5 m^3 m^{-3}, and 0.48 m^3 m^{-3}, respectively. The simulation showed that the electrode did not use more than 10% of the total amount of oxygen consumed from the chamber and that no concentration gradient was induced in the soil pillar.

Preliminary electrode experiments

Two gases in the soil system may seriously interfere with the oxygen measurement, namely N_2O and CO_2. Nitrous oxide may be reduced at a platinum cathode (Johnson and Sawyer 1974; Eberhard and Mindt 1979), resulting in too high a reading.

An experiment, however, in which an electrode equipped with a 6-μm Teflon membrane was exposed to mixtures containing volume fractions of O_2 (0, 0.05, 0.1, and 0.2) and N_2O (0, 0.2, 0.4, and 1.0) in N_2 prepared in a 30-ml gas syringe showed a maximum relative increase of about 4% at an O_2-N_2O-N_2 mixture of 0.2-0.4-0.4. Another experiment showed that polarograms (from −0.3 to −1.2 V) prepared in O_2-N_2O-N_2 mixtures containing volume fractions of 0.1-0.2-0.7 and 0.1-0.0-0.9, respectively, were similar, and an interference peak of N_2O around −0.7 V, as reported by Eberhard and Mindt (1979), was not found.

The other gas, CO_2, may reduce oxygen measurements (Küchler et al. 1978). Carbon dioxide may lower the pH in the buffered electrolyte that electrically bridges the cathode and anode. This could result in a less efficient reduction of hydrogen peroxide (Kreuzer et al. 1980) and thus in a (partial) shift from a four-electron oxygen reduction reaction to a two-electron reaction (Lee and Tsao 1979).

To evaluate this effect, an electrode was exposed to O_2-CO_2-N_2 mixtures (prepared as described above) with volume fractions of 0.2-0.25-0.55 and 0.2-0.5-0.3, respectively. The electrode signal at the 0.25 CO_2 fraction showed a maximum relative decrease of 7% after 12 min; it finally (after 2 h) stabilized at this level after an initial recovery. The electrode signal at the 0.5 CO_2 fraction showed a very strong decrease that did not recover.

It is concluded that the electrodes can be used in soil, as the extreme conditions imposed with respect to the volumetric gas fractions will probably not be reached. Moreover, in the soil experiments a 12-μm membrane is used.

Calculations

Oxygen pressure in the pores of the soil aggregate at the two locations during an experiment was calculated assuming that the electrode signal had a linear drift with time. The measured oxygen pressure was not converted to oxygen concentration, because it can be used directly to check the simulation model.

Water content

Technical description

Water content is measured along the diameter of the experimental soil aggregate by means of 60-keV gamma radiation from ^{241}Am. Theory and application of attenuation methods are described by Gardner (1965) and Gardner and Calissendorff (1967). This technique can safely be used here, because the radiation energy of the ^{241}Am source is very low compared with the energy necessary to kill or inactivate microorganisms (Becking 1971)[3]. The source-detector system used was developed by the Department of Soil Science and Plant Nutrition at the Agricultural University, Wageningen. The system has been described by de Swart and Groenevelt (1971). The transport mechanism to locate the rigid support holding the source and detector (Fig. 2), however, was newly built for the present study. This transport mechanism consists of two stainless steel bars along which the support is guided with ball bushings. Locating is done by a motor-driven precision screw-thread that can be rotated in a female screw attached to the rigid support (detailed drawings available on request). The support can automatically be located at each millimeter or at regular intervals along the diameter of the soil container.

At a source-detector distance of 7.2 cm, typical corrected count rates through air, empty soil container, oven-dry soil, and nearly water-saturated soil are 35 250, 14 850, 5450, and 4350 counts per second, respectively.

Comment on procedures (Table 1, items 4 and 15)

The counting durations for calculating instrumental dead time (see below), attenuation coefficient of water, reference scans at initial water content, and scans through wetted soil were 30, 30, 60, and 18 s, respectively.

Data are converted to counts per second, corrected for instrumental dead time according to Overman and Clark (1960), and corrected for drift in the electronics (less than 0.5% and not time dependent) using the reference measurements. Instrumental dead time ($2.87\ 10^{-6}$ s per count) was found by a procedure described by Fritton (1969), from which the weighed variance was omitted, however.

The attenuation coefficients of water and soil are needed to calculate water contents and bulk densities. For water it was 0.190 $cm^2\ g^{-1}$, assuming a density of 1 g cm^{-3}. This number compared well with the value of 0.196 $cm^2\ g^{-1}$ given by Groenevelt et al. (1969). The attenuation coefficient for the sandy loam soil was determined as 0.280 $cm^2\ g^{-1}$.

Water contents located symmetrically around the center of the soil container were considered duplicates: when corresponding water contents were plotted against each other, slopes of about unity and intercepts of about zero were obtained. This indicates that the changes in water contents in the soil aggregate were small during the determination of a scan.

The distribution of the volumetric water content in space and time can directly be compared with the results of the simulation model.

Chemical analyses

Comment on procedures (Table 1, items 10, 11, 6, and 22)

The soil is wetted with a solution that may contain potassium nitrate (KNO_3) and/or glucose ($C_6H_{12}O_6$). Concentrations are so chosen that equivalents of about 65 kg NO_3^-N and 65 kg C per ha are applied to the aggregate.

Nitrate, nitrite, and ammonium are determined in the extract of the soil-KCl mixture used to measure pH. In the nitrate determination, NO_3^- ions are routed through a Cu-Cd

[3] In the worst case, that all microorganisms are situated just above the soil container bottom, that living mass and density of microorganisms are 0.014% of soil mass (derived from Woldendorp 1981) and equal to the density of water, and that the radiation period is 10 min, the radiation absorbed dose is $2.6\ 10^{-4}$ Gy. This number should be compared with D-10 values (radiation dose producing 10% survival or 90% lethality in microorganisms) of the order of 100 Gy (Becking 1971).

reduction column to form nitrite. This ion is converted into a colored organic complex of which the concentration is measured spectrophotometrically (Novozamsky et al. 1983). Nitrite is determined similarly, but now the reduction column is left out of the measuring system. The determination of ammonium is described by Novozamsky et al. (1974).

Calculations

Chemical data were expressed on an oven-dry basis. The nitrogen species, i.e., NO_3^-, NO_2^-, NH_4^+, N_2O, and N_2, were used to calculate the inorganic N balance at the start and the end of an experiment. The difference between the amounts of nitrate before and after an experiment does not necessarily balance with the sum of the amounts of the produced inorganic nitrogen species, because mineralization and/or immobilization of nitrogen may take place.

Error analysis

State variables obtained from experiments result from a combination of a number of differently measured quantities. Reliability of the state variables is assessed by calculating the propagation of the errors in the measured quantities on these final results.

Systematic errors in the measurements are avoided as much as possible by calibrating the various measuring instruments. Random errors, however, remain in each measured quantity. They can be evaluated from knowledge about the measuring instruments. Error propagation is calculated by procedures outlined by Berendts et al. (1973), using partial derivatives with respect to each measured quantity. These calculations yield confidence intervals of one standard deviation.

The equation to calculate water content from gamma-radiation data appeared too complex to obtain the partial derivatives analytically. Therefore, these partial derivatives were evaluated numerically.

The measurements with the polarographic oxygen electrodes yield electrical signals that were converted to oxygen pressures, using calibration lines before and after an experiment. These calibration lines, obtained by linear regression, were also used to calculate confidence limits for oxygen pressure estimates, using Eq. (6.14.2) of Snedecor and Cochran (1974). Errors in the oxygen measurements in the period between determinations of calibration lines were calculated by linear interpolation.

Values for the errors in individual measurements used in the error propagation calculations are summarized in Table 2. No errors have been assumed in time, molecular weights, and locating the gamma-radiation apparatus.

Errors are indicated as vertical bars in Figs. 4 through 7, when they are larger than the symbols used.

RESULTS AND DISCUSSION

One representative coherent data set on the distribution of water, gases, and nitrogen species is presented in Figs. 4 through 7 and Table 3 for

TABLE 2

Values for errors in individual measurements for error propagation calculations

Measurement	Error	Dimension
Gas chromatography[a]	1.5	%
Particle density[b]	1	%
Chemical analyses[c]	10	%
Volume of gas collection circuit	5	ml
Burette readings	0.2	ml
Amount of wetting solution	2	ml
Concentration of KNO_3 and $C_6H_{12}O_6$ in wetting solution	0.05	$g\ L^{-1}$
Weight of initially moist soil	2	g
Initial gravimetric water content[d]	0.001	$g\ g^{-1}$
Final gravimetric water content[e]	0.003	$g\ g^{-1}$
Diameter of concentric layers (measuring tape, MT)	0.1	cm
Height of sample (vernier callipers)	0.02	cm
Water penetration depth (MT)	0.1	cm
Dead time	$0.05\ 10^{-6}$	s
Water density	0.0001	$g\ cm^{-3}$
Gamma radiation measurement	$\frac{counts^{1/2}}{count\ time}$	s^{-1}

[a] Statistical analysis (SA) of calibration chromatograms.

[b] Based on analysis of method of determination.

[c] Based on analysis of number of data at two locations in layers of concentrically partitioned aggregate.

[d] SA of quadruple determination.

[e] SA of duplicate determination.

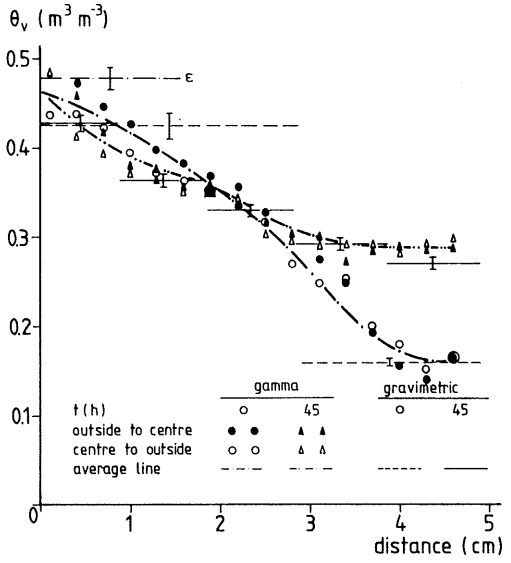

FIG. 4. Volumetric water distribution in soil aggregate (center at distance 4.9 cm) at start and end of experiment.

Lelystad sandy loam soil. In a forthcoming paper other data sets will be discussed in conjunction with the simulation model.

The average density of the Lelystad aggregate was 1.38 ± 0.03 g cm^{-3}. Variation in densities determined with gamma radiation was well within 5% of the average.

Figure 4 shows the volumetric water distribution at the start of the experiment and at the end, after 45 h. The water volume should be conserved, because no evaporation of water from the aggregate takes place. The volume of water was obtained by summation of the amounts of water in 49 concentric rings of 1-mm thickness each. The water content of each ring was obtained from linear interpolation between the averaged measured water contents. This yielded an initial water volume (V) of 71.2 ± 0.6 ml. Following the same procedure at 45 h gave V = 72.0 ± 0.6 ml. From the gravimetric moisture measurements on the five concentric rings at 45 h, V = 71.0 ± 0.7 ml was calculated. The close agreement among these data shows that the methods to measure water content are satisfactory.

After about 24 h water no longer redistributed (data not shown), even though a homogeneous volumetric water content (0.363 ± 0.008) was not attained. This indicates that a homogeneous suction distribution was attained in the soil aggregate combined with a heterogeneous water distribution due to hysteresis in the soil water characteristic. In the absence of hysteresis, the air-filled porosity at equilibrium (0.115 cm^3 cm^{-3}) would have been sufficient to let oxygen diffuse into the aggregate and to stop anaerobic processes. In the presence of hysteresis, however, a small amount of added water is sufficient to decrease gaseous diffusion to such an extent that anaerobiosis may occur in soil.

The volume of air-filled pores in the outer shell of the aggregate remained close to zero, even after 45 h. Thus the exchange of gases between the soil and the gas collection circuit took place through the water phase, and it was expected that the aggregate remained anaerobic after the oxygen in its interior was consumed.

Figure 5 confirms this expectation. It shows oxygen pressure in the aggregate center to equal zero after 13 h, while the peripheral oxygen pressure remains about 2 kPa. Outside the aggregate, however, and after 45 h, the amount of oxygen had decreased by only 19.5 ml (Fig. 6), which agrees with an oxygen pressure of 15.7 kPa. Oxygen flux can be obtained by differentiating Fig. 6 with respect to time. Oxygen flux density is obtained by dividing this quantity by

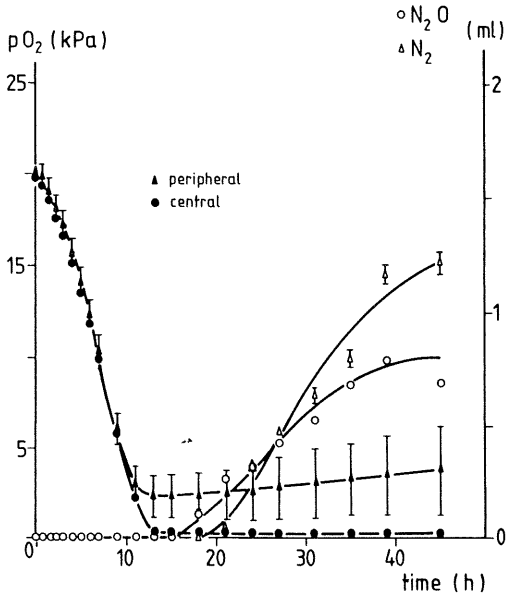

FIG. 5. Oxygen pressure (left y axis) in center and 4 cm from center (peripheral) of soil aggregate and volumes of nitrous oxide and molecular nitrogen in gas collection circuit (right y axis) as function of time.

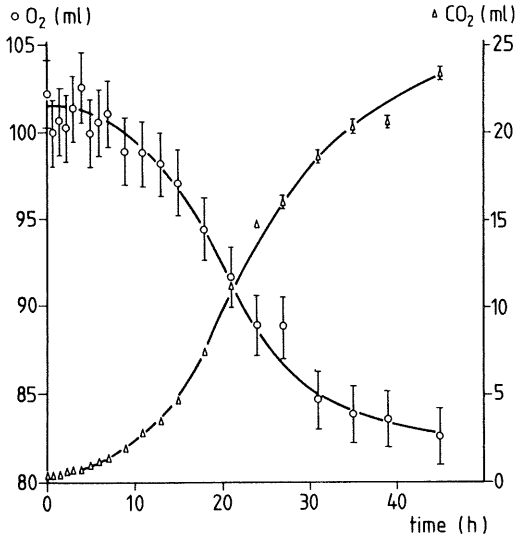

FIG. 6. Volume of oxygen and carbon dioxide in gas collection circuit as function of time.

the surface area of the aggregate (79.7 ± 1.0 cm^2). At 21 h the oxygen flux density amounted to -2.7 ± 10.8 L O_2 m^{-2} d^{-1} (the large confidence interval is discussed below). This value compares favorably with field measurements in bare soil (Russell 1973).

Figure 6 also shows the development of carbon dioxide. The oxygen and carbon dioxide curves form complementary S-shaped curves. The amounts of oxygen consumed (20 ± 3 ml) and carbon dioxide produced (23 ± 0.5 ml) in 45 h were practically the same. This resulted in a respiratory quotient, defined as the ratio of the volumetric rates of carbon dioxide production and oxygen consumption, of about 1. In a partially anaerobic soil, however, only a portion of the soil is consuming oxygen, while the whole is producing carbon dioxide, and the respiratory quotient should exceed 1. Therefore, the respiratory quotient is not a sensitive measure to decide whether a soil is partially anaerobic. Russell (1973) and Gliński and Stępniewski (1985), however, state the opposite with regard to the sensitivity of the respiratory quotient. The discrepancy between a respiratory quotient of about 1 and the knowledge that soil is partially anaerobic can, at least in part, be explained by the differences in solubility of O_2 and CO_2 in soil water (0.033 and 0.946 ml ml^{-1}, respectively, at 100 kPa and 293 K; Hoeks 1972): when CO_2 is produced at the same rate as O_2 is consumed, but is released slower, the respiratory quotient will be underestimated as long as no steady rates of exchange of CO_2 and O_2 are established. A possible correction for solubility differences for the production volumes of O_2 and CO_2 is severely hampered by the unknown concentration gradients of these gases inside the aggregate. The CO_2 curve in Fig. 6 was used to calculate the flux density at 21 h and yielded 3.7 ± 1.3 L CO_2 m^{-2} d^{-1}. This value is of the same order of magnitude as field data of de Jong et al. (1979), though these authors measured CO_2 evolution in a cropped soil. The confidence interval in the O_2 consumption rate is large compared to that in the CO_2 production rate. This is caused by the propagation of the error in the measured amount of O_2, which is determined by the difference of two large numbers. The reasonable error in the measurement of CO_2 makes it a more appropriate measure to assess soil activity.

Assuming that all carbon dioxide comes from the added glucose (4.3 ± 0.2 mmol), and interpreting glucose decay as a first-order rate process, it was calculated that the decay constant is 0.13 h^{-1}. This value is close to the values cited by van Veen and Paul (1981).

Production of nitrous oxide and molecular nitrogen is shown in Fig. 5. These gases occurred in the gas collection circuit a few hours after anaerobiosis was established. The retardation was caused by a combination of adaptation time of the microorganisms to nitrate as new electron acceptor and the time needed for the gas to diffuse through the water phase into the gas collection circuit. Similarly to CO_2, N_2O dissolves well in water (1.1 ml ml^{-1} at 100 kPa and 298 K; Stolzy and Flühler 1978), whereas N_2 has a low solubility (0.016 ml ml^{-1} at 100 kPa and 293 K; Hoeks 1972). This will result in an underestimation of the rate of nitrous oxide development when steady rates are not yet reached, especially in very wet soils. Again, solubility corrections for gas production volumes are hampered for reasons explained above. Furthermore, Fig. 5 shows that the rate of release of N_2 is higher than that of N_2O. This will partly be due to the low N_2 solubility in soil water resulting in an exclusion of N_2 from the water phase. On the other hand, nitrous oxide will be reduced further to molecular nitrogen, which decreases its release. These effects agree with findings reported by Letey et al. (1980).

The N_2O and N_2 flux densities derived from

Fig. 5 equal 1.3 and 1.9 kg N ha^{-1} d^{-1}. Similar values were found in field experiments by Rolston et al. (1976 and 1978), but Colbourn and Dowdell (1984) found lower values for the flux densities in field situations. These flux densities illustrate that a flush of microbial activity in soil is effectively avoided through the procedure of wetting and leaving soil at rest for some time prior to its use, and that the small addition of glucose did not cause an unacceptable increase of the rates of gas production and consumption.

Figure 7 depicts the distributions of nitrate and nitrite in the soil aggregate after 45 h. Large amounts of nitrite were formed, particularly in the outer shell of the aggregate. The nitrate concentration in the central portion of the aggregate (with a diameter of 4.16 cm) had risen from 3.4 to 6.7 mmol kg^{-1} dry soil. In principle, nitrate concentration can be changed by denitrification, dissimilatory nitrate reduction to ammonium (Knowles 1982; Tiedje et al. 1982), mineralization with subsequent nitrification and/or transport of nitrate with the water from the wetting solution. However, since minor amounts of ammonium (about 0.2 mmol kg^{-1} dry soil after 45 h) were found in soil, and nitrification is inhibited in the absence of oxygen (Patrick 1982), dissimilatory nitrate reduction to ammonium, mineralization, and nitrification were not important. A calculation using the initial and final water content data from Fig. 4 and the nitrate concentration in the solution (8.44 g KNO_3 L^{-1}) aplied to the soil yielded a concentration of 7.6 mmol kg^{-1} dry soil. This value is slightly higher than the 6.7 mmol kg^{-1} dry soil actually found: some nitrate may thus have been denitrified. The large amount of nitrite formed in the outer shell of the aggregate must have been caused by the large initial amount of nitrate and by a larger microbial activity due to a greater glucose concentration. Moreover, the accumulation of nitrite may be due to the high pH of the soil, as suggested by Cooper and Smith (1963). The distributions of water, nitrite, and oxygen at the end of the experiment support the statement made in the introduction, that denitrification will occur only when, at a certain place and time, oxygen is absent and bacteria capable of denitrification, water, nitrate, and decomposable organic compounds are present. Therefore, the magnitude of the rate of denitrification will in principle not correlate with the anaerobic volume in aggregates. This was confirmed by the findings of Sexstone et al. (1985).

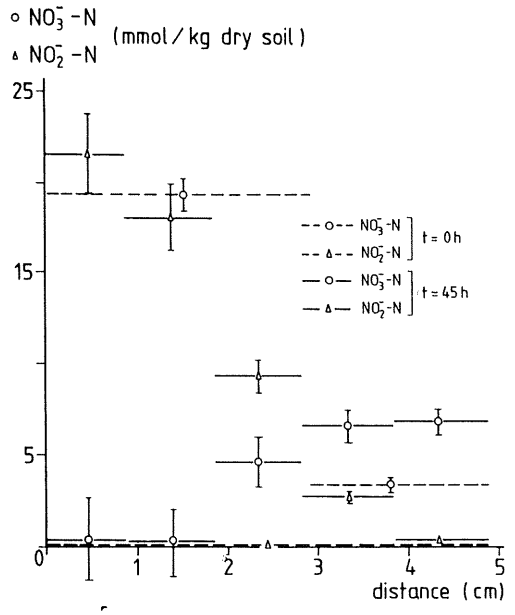

FIG. 7. Distribution of nitrate and nitrite in soil aggregate (center at a distance of 4.9 cm) at start and end of experiment.

TABLE 3

Nitrogen balance of the Lelystad soil

Time, h	0.0	45.0
	mmol	
NO_3^-	4.53	0.64
NO_2^-	0.02	3.93
NH_4^+	0.0	0.05
$N_2O + N_2$	0.0	0.21
	4.55 ± 0.19	4.83 ± 0.36

Table 3 summarizes the molar balance with regard to inorganic nitrogen in the soil aggregate. The last line shows that the N balance is good. The nitrate that has vanished (3.9 ± 0.3 mmol) is quantitatively recovered in the other nitrogen species (4.2 ± 0.3 mmol). Because mineralization, nitrification, and dissimilatory nitrate reduction to ammonium were negligible, it is concluded that denitrification was the sole nitrogen transformation process occurring in the soil aggregate.

CONCLUDING REMARKS

The data presented show that the new type of respirometer system makes it possible to meas-

ure the course in space and time of the state variables that determine whether anaerobiosis and denitrification occur in soil. A full explanation of the relationships among the measured data, however, requires a simulation model that describes the interactions between the active biomass; transport processes of water, gases, and ions; and such storage factors as different solubilities of gases in soil water. Thus, measurements are gathered to evaluate a simulation model, but a simulation model is needed for a full interpretation of measured data.

It is not unlikely that the simultaneous occurrence of water saturation, anaerobiosis, organic compounds, and nitrate at the same place will have a low frequency in field soil. Therefore, it is possible that actual denitrification in field soils does not correlate with potential denitrification, such as measured with anaerobically incubated soil. Techniques like the one reported here are thus needed for a more realistic approach of the dynamics of partial anaerobiosis and denitrification.

The measuring system described is flexible. For instance, water transport may be excluded by saturating the aggregate, so as to avoid the influence of mass flow of water on the results. Then, only diffusion of ions and gases will take place through the water phase, and their submodels may be checked. On the other hand, the atmosphere in the gas collection circuit may exclusively be replaced by neon, so as to study denitrification under fully anaerobic conditions, while other factors affecting denitrification, such as the living biomass and the flow of water, ions, and gases, are maintained. Also nitrite instead of nitrate can be used as an electron acceptor in the soil.

ACKNOWLEDGMENTS

Thanks are due to Prof. G. H. Bolt and his staff of the Department of Soil Science and Plant Nutrition, who have given me the opportunity to carry out the experimental work in their laboratory. I wish to thank the following people who contributed to various parts of the experimental work: H. P. Kimmich, J. G. Spaan, and J. de Koning of the University of Nijmegen for help with the polarographic oxygen sensors; J. G. de Swart and G. L. Jupijn of ITAL for manufacturing the amplifiers for the oxygen sensors; D. P. van den Akker, student at the school for technology at Arnhem (STA), for preparing a manual for these amplifiers; J. D. Wouda (STA) for investigating the influence of nitrous oxide on the performance of the oxygen electrodes; Th. J. Jansen of CABO for manufacturing the soil container and a number of other parts of the respirometer system; M. Schimmel, C. Rijpma, J. van der Goor, and A. van Wijk, who drew and manufactured the support for the gamma system; H. Lindeman (STA) and P. H. F. M. van Twist (STA), for building the power supply for the support, and C. Dirksen and P. Koorevaar for discussions about the gamma measurements; W. Ch. Melger for help with gas chromatographic problems; H. Vrijmoeth (student at the laboratory school RMHAS at Wageningen) for the preliminary experiments to assess the appropriate gas composition for calibrating the gas chromatrograph, and J. Treur (STA) for developing a program to calculate peak areas from gas chromatograms; E. B. Tijssen, Jr. (STA) and H. Lindeman (STA) for programming parts of the error analysis; V. J. G. Houba and A. van den Berg for discussing and performing the chemical soil analysis; and S. Maasland, C. A. Kok, W. van Barneveld, and W. C. Nieuwboer for their assistance in solving many technical problems. The Netherlands Fertilizer Institute (NMI) provided a small subsidy. Prof. C. T. de Wit, Prof. G. H. Bolt, and Dr. B. H. Janssen reviewed the manuscript.

REFERENCES

Allison, L. E., and C. D. Moodie. 1965. Carbonate. *In* Methods of soil analysis, pt. 2. C. A. Black (ed.). Agronomy 9. American Society of Agronomy, Madison, Wis., pp. 1379–1396.

Bascomb, C. L. 1964. Rapid method for the determination of cation exchange capacity of calcareous and non-calcareous soils. J. Sci. Food Agric. 15:821–823.

Becking, J. H. 1971. Radiosterilization of nutrient media. Misc. Papers 9, Landbouwhogeschool Wageningen, The Netherlands, pp. 55–87.

Berendts, B. Th., H. J. A. Blaauw, B. J. M. Harmsen, J. C. Smit, and S. H. Tijs. 1973. Foutenleer en statistiek. Agon Elsevier, Amsterdam, p. 141.

Birch, H. F. 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10:9–31.

Birch, H. F. 1959. Further observations on humus decomposition and nitrification. Plant Soil 11:262–286.

Blackmer, A. M., and J. M. Bremner. 1977. Denitrification of nitrate in soils under different atmospheres. Soil Biol. Biochem. 9:141–142.

Colbourn, P., and R. J. Dowdell. 1984. Denitrification

in field soils. *In* Biological processes and soil fertility. J. Tinsley and J. F. Darbyshire (eds.). Martinus Nijhoff, The Hague, pp. 213–226.

Cooper, G. S., and R. L. Smith. 1963. Sequence of products formed during denitrification in some diverse western soils. Soil Sci. Soc. Am. Proc. 27:659–662.

Currie, J. A. 1961. Gaseous diffusion in the aeration of aggregated soils. Soil Sci. 92:40–45.

Day, P. R. 1965. Particle fractionation and particle-size analysis. *In* Methods of soil analysis, pt. 1. C. A. Black (ed.). Agronomy 9. American Society of Agronomy, Madison, Wis., pp. 545–567.

de Jong, E., R. E. Redmann, and E. A. Ripley. 1979. A comparison of methods to measure soil respiration. Soil Sci. 127:300–306.

Delwiche, C. C. 1981. The nitrogen cycle and nitrous oxide. *In* Denitrification, nitrification, and atmospheric nitrous oxide. C. C. Delwiche (ed.). Wiley, New York, pp. 1–15.

de Swart, J. G., and P. H. Groenevelt. 1971. Column scanning with 60 KeV gamma radiation. Soil Sci. 112:419–424.

Eberhard, P., and W. Mindt. 1979. Interference of anesthetic gases at oxygen sensors. Birth Defects: Orig. Artic. Ser. 15:65–74.

Fatt, I. 1976. Polarographic oxygen sensors. CRC Press, Cleveland, Ohio, p. 278.

Fillery, I. R. P. 1983. Biological denitrification. *In* Gaseous loss of nitrogen from plant-soil systems. J. R. Freney and J. R. Simpson (eds.). Martinus Nijhoff, The Hague, pp. 33–64.

Fritton, D. D. 1969. Resolving time, mass absorption coefficient and water content with gamma-ray attenuation. Soil Sci. Soc. Am. Proc. 33:651–655.

Gardner, W. H. 1965. Water content. *In* Methods of soil analysis, pt. 1. C. A. Black (ed.). Agronomy 9. American Society of Agronomy, Madison, Wis., pp. 82–127.

Gardner, W. H., and C. Calissendorff. 1967. Gamma-ray and neutron attenuation in measurements of soil bulk density and water content. Proc. symp. isotope and radiation techniques in soil physics and irrigation studies, Istanbul (IAEA, Vienna).

Gleichmann, U., and D. W. Lübbers 1960. Die Messung des Sauerstoffdruckes in Gasen und Flüssigkeiten mit der Pt-Electrode unter besonderer Berücksichtigung der Messung im Blut. Pflügers Arch. Ges. Physiol. 271:431–455.

Gliński, J., and W. Stępniewski, 1985. Soil aeration and its role for plants. CRC Press, Boca Raton, Fla., p. 229.

Grable, A. R. 1966. Soil aeration and plant growth. Adv. Agron. 18:57–106.

Greenwood, D. J. 1961. The effect of oxygen concentration on the decomposition of organic materials in soil. Plant Soil 14:360–376.

Groenevelt, P. H., J. G. de Swart, and J. Cisler. 1969. Water content measurement with 60 keV gamma ray attenuation. Bull. Int. Assoc. Sci. Hydrol. 14:67–78.

Hoeks, J. 1972. Effect of leaking natural gas on soil and vegetation in urban areas. PUDOC, Wageningen, p. 120.

Ingraham, J. L. 1981. Microbiology and genetics of denitrifiers. *In* Denitrification, nitrification, and atmospheric nitrous oxide. C. C. Delwiche (ed.). Wiley, New York, pp. 45–65.

Johnson, K. E., and D. T. Sawyer. 1974. The electrochemical reduction of nitrous oxide in alkaline solution. J. Electroanal. Chem. Interfacial Electrochem. 49:95–103.

Kimmich, H. P., and F. Kreuzer. 1969. Telemetry of respiratory oxygen pressure in man at rest and during exercise. *In* Oxygen pressure recording in gases, fluids, and tissues. F. Kreuzer (ed.). Progr. Resp. Res. H. Herzog (ed.), vol. 3. Karger, Basel, pp. 22–39.

Knowles, R. 1982. Denitrification. Microbiol. Rev. 46:43–70.

Kreuzer, F., H. P. Kimmich, and M. Březina. 1980. Polarographic determination of oxygen in biological materials. *In* Medical and biological applications of electrochemical devices. J. Koryta (ed.). Wiley, New York, pp. 173–261.

Küchler, G., W. Wagner, and I. Wolburg. 1978. Experimental study on errors in dynamic measurement of oxygen intake. Biotelemetry 4:109–112.

Lee, Y. H., and G. T. Tsao. 1979. Dissolved oxygen electrodes. *In* Advances in biochemical engineering. T. K. Ghose, A. Fiechter, and N. Blakebrough (eds.). Springer-Verlag, New York, pp. 35–86.

Leffelaar, P. A. 1979. Simulation of partial anaerobiosis in a model soil in respect to denitrification. Soil Sci. 128:110–120.

Letey, J., W. A. Jury, A. Hadas, and N. Valoras. 1980. Gas diffusion as a factor in laboratory incubation studies on denitrification. J. Environ. Qual. 9:223–227.

McNair, H. M., and E. J. Bonelli. 1969. Basic gas chromatography. Varian, U.S.A., p. 306.

Mebius, L. J. 1960. A rapid method for the determination of organic carbon in soil. Anal. Chim. Acta 22:120–124.

Millington, R. J. 1959. Gas diffusion in porous media. Science 130:100–102.

Novozamsky, I., R. van Eck, J. Ch. van Schouwenburg, and I. Walinga. 1974. Total nitrogen determination in plant material by means of the indophenol-blue method. Neth. J. Agric. Sci. 22:3–5.

Novozamsky, I., V. J. G. Houba, D. van der Eijk, and R. van Eck. 1983. Notes on determinations of nitrate in plant material. Neth. J. Agric. Sci. 31:239–248.

Novozamsky, I., V. J. G. Houba, E. Temminghoff, and J. J. van der Lee. 1984. Determination of "total" N and "total" P in a single soil digest. Neth. J. Agric. Sci. 32:322–324.

Overman, R. T., and H. M. Clark. 1960. Radioisotope techniques. McGraw-Hill, New York, p. 476.

Patrick, W. H., Jr. 1982. Nitrogen transformations in submerged soils. *In* Nitrogen in agricultural soil. F. J. Stevenson (ed.). Agronomy 22. American Society of Agronomy, Madison, Wis., pp. 449–465.

Prazma, J., D. Smith, and W. J. Jochem. 1978. Current-to-voltage converter for measurement of oxygen. J. Appl. Physiol.: Respirat. Environ. Ex-

ercise Physiol. 44:977–980.
Rolston, D. E., D. L. Hoffman, and D. W. Toy. 1978. Field measurement of denitrification: 1. Flux of N_2 and N_2O. Soil Sci. Soc. Am. J. 42:863–869.
Rolston, D. E., M. Fried, and D. A. Goldhamer. 1976. Denitrification measured directly from nitrogen and nitrous oxide gas fluxes. Soil Sci. Soc. Am. J. 40:259–266.
Russell, E. W. 1973. Soil conditions and plant growth. Longmans, London, p. 849.
Scholander, P. F. 1942. Volumetric microrespirometers. Rev. Sci. Instr. 13:32–33.
Sexstone, A. J., N. P. Revsbech, T. B. Parkin, and J. M. Tiedje. 1985. Direct measurement of oxygen profiles and denitrification rates in soil aggregates. Soil Sci. Soc. Am. J. 49:645–651.
Smith, K. A. 1977. Soil aeration. Soil Sci. 123:284–291.
Smith, K. A. 1980. A model of the extent of anaerobic zones in aggregated soils and its potential to estimates of denitrification. J. Soil Sci. 31:263–277.
Snedecor, G. W., and W. G. Cochran. 1974. Statistical methods. Iowa State Univ. Press, Ames, p. 593.
Stolzy, L. H., and H. Flühler. 1978. Measurement and prediction of anaerobiosis in soils. *In* Nitrogen in the environment, vol. 1. D. R. Nielsen and J. G. MacDonald (eds.). Academic Press, New York, pp. 363–426.
Tiedje, J. M., A. J. Sexstone, D. D. Myrold, and J. A. Robinson, 1982. Denitrification: Ecological niches, competition and survival. Antonie van Leeuwenhoek 48:569–583.
Thompson, B. 1977. Fundamentals of gas analysis by gas chromatography. Varian, U.S.A., p. 132.
van Veen, J. A., and E. A. Paul. 1981. Organic carbon dynamics in grassland soils: 1. Background information and computer simulation. Can. J. Soil Sci. 61:185–201.
Woldendorp, J. W. 1981. Nutrients in the rhizosphere. Agricultural yield potentials in continental climates. Proc. 16th Coll. Int. Potash Institute, Bern, pp. 99–125.