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ABSTRACT

SOIL DENITRIFICATION: CONTROL BY OXYGEN
AND MOISTURE AND MEASUREMENT IN THE FIELD

by

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Denitrification is known to cause loss of combined nitrogen from agricultural soils, however methodological limitations have made realistic measurement of these losses under natural conditions very difficult. In this study a soil core method using the acetylene inhibition of nitrous oxide reduction was developed to measure field denitrification rates in agricultural soils. Recirculation of the pore space gases in a closed system allowed rapid distribution of acetylene to active denitrification sites and equilibrium of the N_2O produced with the recirculating gaseous phase. Denitrification rates could be measured on a soil core within 2 hours which allowed many replicate samples to be evaluated and variability of the mean rate estimate described. Nitrogen losses were determined to be 11.6 $kg\text{-}N \cdot ha^{-1} \cdot mo^{-1}$ for an aggregated clay loam soil and 6.1 $kg\text{-}N \cdot ha^{-1} \cdot mo^{-1}$ for a largely unaggregated sandy loam soil. The response of soil denitrification to increased moisture was compared in the two soils. Increased denitrification rates were observed in both soils following water inputs of at least 1 cm following irrigation or rainfall. The denitrification rate in the sandy loam soil began to increase immediately after water addition and reached a maximum rate within 3 to 5 hours. Denitrification rates returned to preirrigation levels within 12 hours. A similar, but slower denitrification response occurred in the heavier textured clay loam soil; 8 to 12 hours elapsed before a maximum rate was observed, and 48 to 60 hours was required before the original rate was

restored. Peak field losses of 1.1 and 1.9 kg-N$\cdot$ha$^{-1}\cdot$day^{-1} occurred following water inputs of 7 and 4 cm, corresponding to air filled porosities of 37 and 30% in the sandy loam and clay loam soils, respectively. Nitrogen losses from the clay loam soil were double that of the sandy loam soil although the sandy loam soil received almost twice the water input, reflecting the difference in the temporal duration of the period of highest N-loss.

In laboratory studies denitrification rates increased with increasing moisture content both in the presence and absence of oxygen. The increased aerobic rate was attributed to increased anaerobic microsites due to decreased oxygen diffusion in the wetter cores. Increased anaerobic rates were attributed primarily to increased substrate availability, since no increase in denitrifying enzyme content could be detected. Cores collected after irrigation exhibited a greater response to rewetting than did dry cores wet to the same moisture content. Denitrification rates followed a hyperbolic relationship with pore space oxygen concentrations. This relationship depended on soil moisture content. Wetter cores exhibited a higher percentage of the potential anaerobic rate at a given oxygen concentration when compared with drier cores. A clay loam soil achieved 10% of the potential anaerobic rate at higher pore space oxygen concentrations when compared with a sandy loam soil at the same air filled porosity. Oxygen diffusion coefficients that provided the best fits to the experimental data were estimated from a model predicting soil anaerobiosis.

An oxygen microelectrode was modified to measure oxygen concentrations in wet aggregates of a silt loam soil. The microelectrode had a sensing tip area of 3 μm, and oxygen measurements

could be made in as little as 0.1 mm increments to a depth of 12 mm. When aggregates were incubated in air, steep oxygen gradients usually occurred over very small distances from the aggregate surface. The smallest aggregate exhibiting a completely anaerobic center had a radius of 4 mm, although small aggregates (radius $\leq$ 6 mm) were generally oxic. Larger aggregates (radius $\geq$ 10 mm) often had measurable anaerobic centers, with the exception of those from a native prairie soil which exhibited irregular oxygen profiles, apparently due to oxygen intrusion caused by old root channels. Oxygen profiles obtained in 45 degree increments around an aggregate circumference were used to construct contour maps of oxygen concentrations within the aggregate. Oxygen gradients were often assymmetric, suggesting non-uniform oxygen consumption. An average oxygen diffusion coefficient of 8.5×10^{-6} $cm^2 \cdot s^{-1}$ was determined for saturated aggregates. The aggregate anaerobic radii, calculated assuming radial diffusion, were similar to those measured directly with the electrode. Anaerobic centers were present in all aggregates that denitrified, but not all aggregates with anaerobic zones denitrified. The denitrification rate did not correlate with the size of the anaerobic zone, indicating that factors other than anaerobic volume alone determined the observed rates.

To Julie and Sean

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INTRODUCTION

Intensive nitrogen fertilization to maximize the yield of agronomic crops did not become prevelant in this country until after the second world war (Scarseth, 1942). Ten years later Allison (1955), reported the "enigma" that nitrogen inputs could not be accounted for by measured nitrogen outputs from these systems. Based on this unaccounted for nitrogen, he suggested that between 10 to 30% of the nitrogen inputs were lost from soil due to denitrification. The following year, Hauck and Melstead (1956) discussed some of the methods available at that time to measure denitrification in the laboratory, and concluded that the rates measured under these artificial conditions were very high when compared with projected field losses. In the 30 years since these reports, progress has been made on the basic physiology and biochemistry of denitrifying bacteria, however, quantitative direct measurements of denitrification losses from soils have remained elusive. Lack of this information necessitates the currently practiced strategy of routine over-fertilization. This represents an economic loss to the farmer, and can cause increased nitrate pollution of groundwater (Keeney, 1982). Increased fertilizer use may also increase N_2O emissions, which have been implicated in stratospheric ozone depletion, and gradual warming of the planet (McElroy et al., 1977; Wang et al., 1976). The early observations of Allison and Hauck pose a question that is central to this dissertation, i.e. how can denitrification rates be reliably measured to obtain good estimates of denitrification N-losses in the field, and what environmental factors control these losses?

In this introduction I will not attempt to review all aspects of

denitrification; indeed the many lines of current active research make this a prohibitive task. The interested reader is referred to several recent reviews that cover advances in this rapidly changing area of investigation: Payne, (1973); Delwiche and Bryan, (1976); Stouthamer, (1976); Focht and Verstraete, (1977); Rolston, (1981); Ryden, (1981); Payne, (1981); Knowles (1981,a,b; 1982); and Firestone(1982). I will briefly review literature pertaining to environmental factors observed to control denitrification rates in soil, as well as methodological approaches that have been used in attempts to measure field denitrification N-losses from soil systems.

Denitrifying bacteria are facultatively anaerobic heterotrophs that in the absence of O_2 can use NO_3^- and NO_2^- as alternate terminal electron acceptors, and stoichiometrically reduce these compounds to the gaseous products, N_2O and N_2. The latter is generally the dominant product from soil, although factors have been identified which increase the proportion of N_2O produced (Firestone et al., 1980). When O_2 is available these organisms preferentially live as aerobes. Oxygen is known to both repress synthesis of denitrifying enzymes, and to inhibit the activity of these enzymes once synthesized, therefore denitrification must occur when the soil is anaerobic (Knowles, 1982). The macropores of soil, unless completely saturated with water are generally found to contain O_2 at concentrations near those of the atmosphere (Parkin and Tiedje, 1983). Anaerobic zones in soil are thought to occur only as a result of active O_2 consumption by bacteria, fungi, and plant roots. The rate of O_2 consumption must exceed the rate of O_2 supplied by diffusion for anaerobiosis to occur. The rate of O_2 supply in turn depends on the thickness of the water film on soil

particles, since oxygen diffuses at a rate 10,000 to 100,000 times slower through water than through air. The potential respiratory demand of soil heterotrophs, as well as potential activity of soil denitrifiers, also depends on the availability of readily utilizable carbon, and in the latter case, the supply of NO_3^-. Methods to measure natural denitrification rates must maintain the physical structure of the soil since this controls the extent of anaerobiosis and the substrate supply to potential active sites of denitrification. The presence of anaerobic microsites in soil is discussed further in Chapter III of this dissertation.

Most previous studies of environmental factors controlling denitrification rates have been performed using long term incubations (weeks) with soils that often have been air dried and sieved such that all naturally occurring spatial arrangements within the soil are destroyed. Such artificial homogeneity make extrapolations to the field situation equivocal, but they have been useful in identifying important controlling parameters. It has been generally observed that increasing the soil moisture content, or decreasing the oxygen content of the incubation increases the denitrification rate observed. Various measures of the soil water and oxygen status have been used in an attempt to describe critical levels below which denitrification ceases to occur. Estimates of such critical moisture tensions include 33 kPa (Pilot and Patrick, 1972; Bremner and Shaw, 1958); 25 kPa (Ryden et al., 1979), and 10 kPa (Rolston et al., 1978). Other types of measurements that have been found useful include soil mositure content (Burford and Stefanson, 1973); air filled porosity (Pilot & Patrick, 1972); percent pore space oxygen (Wijler and Delwiche, 1954); percent water holding

capacity (Nommik, 1956; Bremner and Shaw, 1958) and redox potential (Bailey and Beauchamp, 1973). As pointed out by Papendick and Campbell (1978), diffusion of a gas in soil is directly related to water content for all soil textural classes and not to the energy status of the water in the system; the latter is described by moisture tension. I have used water content or percent air filled porosity to describe the water status throughout this dissertation.

Denitrification rates have been observed to increase with increasing nitrogen fertilization rates (Broadbent and Carlton, 1980). They have also been positively correlated with both "readily available" and mineralizable carbon (Burford and Bremner, 1975); exogenous carbonaceous inputs such as manure (Rolston, 1978), or plant materials (Brar et al., 1978); and with association with the plant rhizosphere (Smith and Tiedje, 1979a).

Soil nitrogen budgets provided the earliest estimates for and are still important indirect measures of field denitrification losses (Allison, 1966; Legg and Meisinger, 1982). Reported losses vary from 0 to 70% of the applied fertilizer with an average of 20 to 30%. The general stratagy is to measure the nitrogen pools present in a soil and to assess inputs and exports over a period of time before constructing a balance. Nitrogen unaccounted for by difference is used as the denitrification estimate. Estimates of denitrification by this difference method reflect the cumulative errors of measuring all components of the balance. The transient nature of mineralization and immobilization reactions, difficulties with determining accurate leaching losses, and analytical difficulties in accurately measuring total N pools are a few of the problems encountered in constructing an

accurate balance.

The sensitivity of the balance approach can be increased by labelling a specific nitrogen pool with nitrogen enriched or depleted in ^{15}N. Use of ^{15}N for such studies has been reviewed by Hauck and Bremner (1976). As pointed out by Legg and Meisinger, use of ^{15}N in budget studies is not equivalent to budgets where only unlabelled pools are measured. The former approach focuses on the total N-cyle of the system. The latter is a measure of how the label interacts with the system by tracing its fates through the various pools. It is possible to account for ^{15}N fertilizer lost from a system, but it is difficult to access total denitrification by this method. Problems include heterogeneity of label distribution, the necessity of accurate time dependent measurement of ^{15}N ratios of N pools, and the many assumptions necessary for the varying fates and recycling of N. It may be possible to obtain improved denitrification rates by this method, if analysis employing simultaneous estimation of major N-cycle rate processes is employed (Tiedje et al., 1981; Myrold and Tiedje, 1982).

Denitrification losses estimated by difference contain much uncertainty, and require long term experiments to be accurate. Studies of this kind cannot elucidate short term temporal responses that determine when important nitrogen losses occur. It would be desirable therefore to directly measure the process. Substrate disappearance cannot be used in field situations, since nitrate has assimilatory and dissimilatory fates other than denitrification (Tiedje et al., 1981). Monitoring the appearance of the terminal product is complicated by the presence of an atmospheric background of 78% N_2. One solution has been to follow $^{15}N_2$ and $^{15}N_2O$ appearance from highly enriched $^{15}NO_3^-$ by mass

spectrometry. This approach was used by Rolston et al. (1976; 1978) who added large quatities of this isotope as fertilizer to replicated 1 m^2 field plots, and monitored ^{15}N flux by sampling gas accumulation under plexiglass covers placed on the soil surface for 1 to 3 hours. He observed increasing N-flux with increasing water content, soil temperature, fertilizer application rate, and manure addition. For plots near saturation with high manure additions, fluxes of 70 kg-N$\cdot ha^{-1}\cdot day^{-1}$ were reported, representing a 70% fertilizer loss over a 1 month period. The lowest loss observed at 23^{o} C was 2.5 kg-N$\cdot ha^{-1}\cdot day^{-1}$ from uncropped sites. These results contrast those of Mosier et al.(1982) who could detect no denitrification losses using this method from his sites. Problems with this technique include the high cost of highly enriched ^{15}N and the high application rates necessary to detect sufficient quantities of N-gas for analysis by ratio mass spectrometry. This latter consideration makes this technique inappropriate in situations where the natural pool is normally low, as in most unfertilized sites. An alternative to ratio mass spectrometry is the use of gas chromatography coupled with quadripole mass spectrometry which has a much improved detection limit for $^{15}N_2$ (Focht et al., 1980; Focht and Stolzy, 1978).

Denitrified nitrogen is unavailable for assimilation, whether it remains in the soil macropores or has escaped to the atmosphere. A general criticism of cover techniques is that a flux as opposed to the actual rate of loss is measured. Flux measurements reflect only the rates of gaseous diffusion from the soil matrix to the atmosphere rather than actual denitrification rates. In wet soils, long lag periods can obscure short term temporal responses. As much as 70% of the gas produced can remain in the soil matrix under these conditions (Rice and

Smith, 1982). A further underestimation occurs if downward diffusion is ignored. This later point has been considered in recent measurements by Verbruggen and Vlassak (1983), however measuring this component significantly increases the effort required to obtain a flux estimate, decreasing the number of replicate measurements that are possible.

A alternative approach to addition of $^{15}NO_3^-$ suggested recently is the use of a gas lysimeter to measure dilution by denitrification of added $^{15}N_2$ (Limmer et al., 1982). This method is attractive since no disturbance of natural N pools is required. However the technique requires that the natural N_2 background be reduced by flushing with an inert gas, and requires high production rates before sufficient dilution occurs for detection. This gas lysymeter is a sophisticated apparatus, and does not easily lend itself to the replication necessary to obtain good field loss estimates.

The radioactive isotope ^{13}N provides for extremely sensitive N_2 detection (Tiedje et al., 1979), however cannot practically be used in the field since the half life is 10 minutes and requires a cyclotron or Van de Graff generator to produce. However, this technique has attractive features for some laboratory studies, particularly those with natural samples with low pool NO_3^- sizes. Promising initial rate measurements have recently been performed in this laboratory utilizing a direct injection technique (Jorgensen, 1978) with undisturbed cores of forest soils (G. P. Robertson, personal communication).

Gas chromatography is another major technique used to measure rates of product formation from denitrification. N_2 can be detected directly if background N_2 is removed with an inert gas such as argon or helium, (Burford and Stevanson, 1973), however this approach is insensitive. The

product N_2O can be measured with much greater sensitivity (30 ppb detection limit) using ^{63}Ni electron capture gas chromatography. The observation that low concentrations of acetylene (0.01 to 0.1 atm) inhibits the nitrous oxide reductase of denitrifying bacteria, causing stoichometric accumulation of N_2O from NO_3^- has greatly increased the applicability of this approach (Balderston at al., 1976; Yoshinari and Knowles, 1976). Acetylene inhibition of N_2O reduction has been used in laboratory studies to measure denitrification in soil (Yoshinari et al., 1977; Klemedtsson et al., 1977; Smith et al., 1978), and the technique provides the basis of recent attempts to measure field denitrification rates. Use of this method requires that several cautions be observed: the blockage has been observed to fail in soils after long term incubations (160 hours) by Yeomans and Beauchamp (1978); sufficient concentrations of acetylene (>10%) must be used in situations where the nitrate pool is less than 1 $\mu g\text{-}N \cdot g^{-1}$ (Smith and Tiedje, 1979b); commercial acetylene contains contaminants such as acetone which, unless removed, can increase denitrification rates in incubations of longer than 3 to 5 days (Gross et al.,1982); and most importantly, acetylene has been observed to inhibit nitrifying bacteria and their activity in soil (Hynes and Knowles, 1980; Walter et al., 1979). These considerations necessitate that the acetylene inhibition technique be used in relatively short term incubations, and in situations were the indigenous nitrate pool is in sufficient supply.

Applications of the acetylene inhibition technique in the field have used two main approaches; cover methods, and use with soil cores. With cover methods, acetylene is added the soil by diffusion and N_2O is collected under a cover as it diffuses out. The most extensive studies

reported to date are those of Ryden et al. (1979a,b) and Ryden and Lund (1980) who performed experiments on several irrigated California soils used for vegetable production. They allowed acetylene to diffuse into the sampling area surrounding injection tubes. A cover was then placed over the area and flux measurements determined by sweeping the headspace within the cover through a molecular sieve trap. N_2O could be removed from the traps for analysis by gas chromatography by displacement with water. They observed rates between 7.9 and 19.5 kg-N$\cdot$ha$^{-1}\cdot$mo^{-1} with fertilizer losses ranging from 14 to 52%. Using a variation of this approach, Duxbury et al. (1982) observed losses of only 1.5 kg-N$\cdot$ha^{-1} over a 3 month period.

The other major application of the acetylene inhibition technique involves removal of undisturbed cores from the field for incubation under controlled conditions in the presence of acetylene. Rice and Smith (1982) have described a method in which acetylene saturated water is added to the surface of soil cores prior to incubation to help facilitate acetylene distribution. Acetylene amended air is then passed over the top of the core and the N_2O concentration of the outflow is measured. With knowledge of a gas flow rate, a flux from the core surface can be calculated. This method has been used in a comparative study of soils subjected to minimum tillage techniques which exhibited greater N-losses than conventionally tilled soils. A second approach is to place the core sample within a second closed container into which acetylene is injected and allowed to passively diffuse into the sample. The denitrification rate is monitored by following N_2O accumulation in the closed container over a period of 24 hours (Aulakh et al., 1982; Svensson et al., 1980). Using this method Aulakh et al. observed losses

of 0.5 to 1.5 kg-N$\cdot$ha$^{-1}\cdot$mo^{-1} estimated from once weekly samples. Svensson et al. measured high losses of 30 to 60 kg-N$\cdot$ha$^{-1}\cdot$mo^{-1} from fertilized barley. These methods may suffer from a similar criticism to cover methods, i.e the rate measured is likely to reflect the physical rate of N_2O diffusion rather than the biological rate of production. This is particularly true since the rate also reflects the speed and completeness with which acetylene can diffuse to active sites of denitrification.

In this laboratory, we have developed an acetylene soil core system that addresses these criticisms, and provides additional versatility for experimental evaluation of factors controlling the observed rate of denitrification (Parkin et al, 1983). Use of this method to determine denitrification N-losses from agricultural soils, as well as to describe short term temporal control of denitrification losses is described in Chapter I. Chapter II further addresses the interactive control of denitrification rates by soil moisture content and oxygen concentration, and relates this control to an existing model which describes the extent of soil anaerobiosis. Finally, Chapter III reports direct measurement of anaerobic microsites in soil, and discusses these results with respect to observed denitrification rates.

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CHAPTER I

TEMPORAL RESPONSE OF SOIL DENITRIFICATION RATES TO RAINFALL AND IRRIGATION

The rate of nitrogen loss from soil due to denitrification is thought to be increased by factors that increase the extent of anaerobic sites in the soil (Firestone, 1982). Increasing soil moisture acts as one such factor by decreasing the rate of oxygen diffusion through the soil matrix, allowing the development of anaerobic microsites as oxygen consumption exceeds the rate of its diffusive supply. Several laboratory studies have demonstrated increased nitrogen loss with increasing moisture content from soils incubated in the presence of an aerobic soil atmosphere (Nommik, 1956; Pilot and Patrick, 1972; Baily and Beauchamp, 1973). These studies have generally involved the use of disturbed soils and long term incubations of several weeks. Smith and Tiedje (1979) suggested that it was important to understand the short term denitrification response to increasing soil moisture in order to better characterize denitrification N-losses from field soils following rainfall or irrigation. In limited laboratory studies, they demonstrated that the denitrification rate increased rapidly following a lag period of 6 hours when the soil moisture content of a sandy loam soil was increased from 51% of saturation to 94% of saturation.

McGarity and Rajaratnam (1973) recognized that the denitrification rate in field soils would be controlled by episodic factors such as increasing soil moisture, and suggested that techniques were necessary for accurate short term measurements that preserved the physical control of biological rates imposed by soil structure. Of the techniques

subsequently developed for measurement of denitrification in the field, variations of the acetylene inhibition of N_2O reduction technique (Yoshinari et al., 1977) appear to be the most suitable for these short term field measurements. Artificially irrigated soil cores have been used to compare denitrification as affected by tillage practice on several field sites (Rice and Smith, 1983). No-till soils showed a significantly greater nitrogen flux than conventionally tilled soils, which was attributed to the higher soil moisture content maintained in no-tilled soils. Aulakh et al (1982) made weekly measurements of various field sites using a soil core technique and observed that the denitrification rate was greatly elevated when rainfall decreased the soil air filled porosity (AFP). Ryden et al. (1979), and Ryden and Lund (1980) used a soil cover technique to measure denitrification and reported that the N_2O flux was greatest following irrigation when the %AFP decreased below 22%. In this paper I report the use of a soil core method coupled with acetylene inhibition of N_2O reduction (Parkin et al., 1983a,b) to follow the short term response of the denitrification rate following rainfall or irrigation. The temporal dynamics and duration of this response was examined in both light and heavy textured soils.

MATERIALS AND METHODS

Field Sites

Field sites were established on the Michigan State University farms, East Lansing, Michigan. During May-July and in September, 1981 the experimental areas were located on a Capac clay loam soil (Aeric Ochraqualf) of 34% clay, 30% sand, 36% silt, pH 6.8 (Sites I and II). The field site during May-July, 1982 was a Spinks sandy loam (Psammentic Hapludalf) of 13% clay, 71% sand, 16% silt, pH 6.5 (Site III). The sites were fallow during the experimental periods, and had been planted alternately to corn and soybeans in previous years. Soybeans were harvested from the fall clay loam site just prior to experimentation. Plots were rectangular in shape, approximately 20 m x 10 m. Each site was roto-tilled prior to the start of the experiment. Site I and Site II were fertilized at day 0 with 4.4 kg-N$\cdot$ha^{-1} KNO_3^- and 5 kg-N$\cdot$ha^{-1} KNO_3^-, respectively. Site III was fertilized with 14.2 kg-N$\cdot$ha^{-1} $NH_4NO_3^-$. The sites were chosen for comparison of denitrification rates in fine and coarse textured soils.

During the 44 day experiment at Site I, rainfalls of greater than 1 cm were recorded on days 18, 19, and 27. The site also received a 2 cm irrigation on day 41. Site II was sampled for 19 days and received 1 cm or greater rainfall on days 7, 12, 15, and 17. Site III was sprinkler irrigated at 2 cm or greater on day 5, 15, and 28; and received a 3 cm rainfall on day 16. Water input at each site was determined with a rain gague.

Soil cores 7.6 cm in diameter and 7.6 cm long were collected from both soils for physical characterization. Ten cores of each soil type

Fig. 1 Relationship between air filled porosity and volume percent moisture for a clay loam (squares) and a sandy loam (triangles) soil.

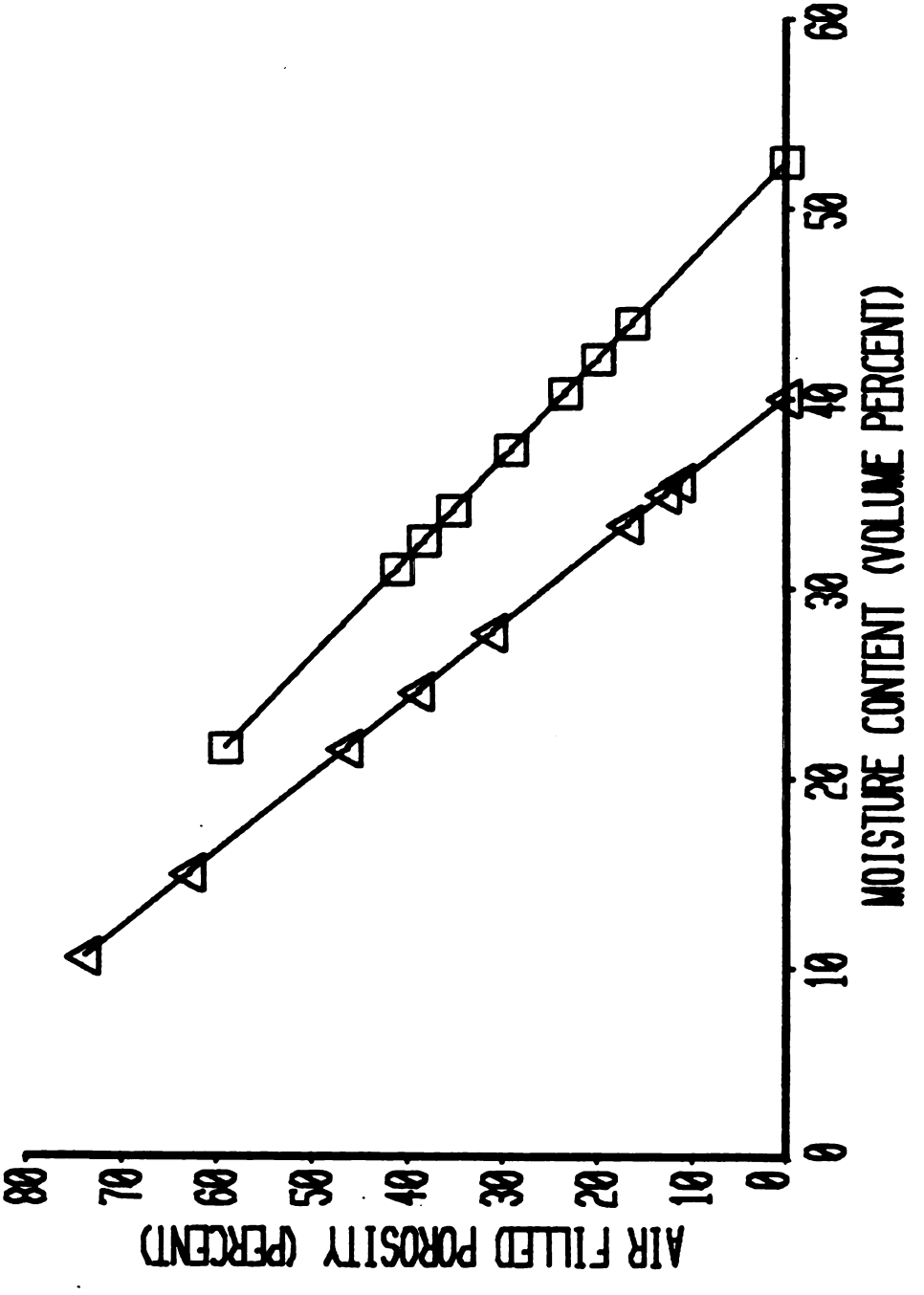
AIR FILLED POROSITY (PERCENT)
80
70
60
50
40
30
20
10
0
MOISTURE CONTENT (VOLUME PERCENT)
0
10
20
30
40
50
60

Figure 1

were saturated in the laboratory and a moisture desorption characteristic determined over a range of 0 to 0.5 MPa. Dry bulk density averaged 1.3 $Mg \cdot m^{-3}$ for the clay loam and 1.7 $Mg \cdot m^{-3}$ for the sandy loam. The relationship of air filled porosity (AFP) to volumetric moisture content for the two soils is shown in Fig. 1. Total porosity was 52% for the clay loam and 40% for the sandy loam soil.

Sample Collection

The field sites were sampled by collecting intact core samples for denitrification rate determinations. Some additional disturbed samples were collected in plastic bags and were refrigerated in the laboratory for later use. The soil cores were collected with a steel corer of 30 cm length and 6.8 cm diameter. A plexiglass or polyvinyl chloride (PVC) core liner (24 cm long) was placed inside the steel corer and held in place with a massive screw cap. The inner diameter of the metal cutting tip and of the core liner was 4.7 cm. The corer was then driven into the ground with a slide hammer. Compaction was usually minimal (0-5%).

Between 4 to 36 cores were collected at Site I nearly every day during the course of the experiment. During the experiments at Site II and III, the sampling regime was altered such that during relatively dry periods cores were collected at 3 to 4 day intervals. After rainfall or irrigation, sampling was increased to intervals of 6 to 12 hours. At each sampling during these experiments, between 12 to 56 cores were collected. After sampling , rubber stoppers were placed in the ends of the core liners which were returned immediately to the laboratory.

Determination of Field Denitrification Rates

Denitrification rates were determined by measuring N_2O accumulation using a variation of the acetylene inhibition of nitrous oxide reduction technique (Yoshinari et al., 1977). This method is described in detail

Fig. 2 Gas flow system used to determine denitrification in soil cores. A, moisturizing flask; B, on-off valve; C, quick-fit connector; D, valving system in gas chromatograph; E, quick-fit connector.

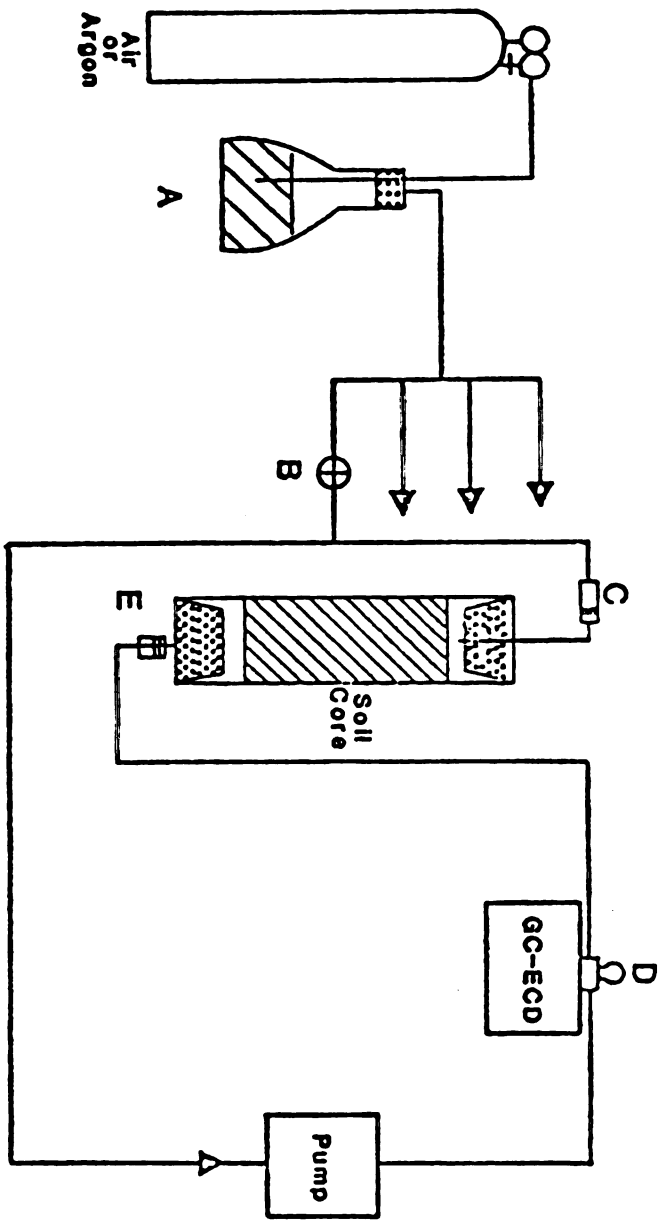

Figure 2

by Parkin et al. (1983a). Acetylene enriched air was recirculated through the pore spaces of the soil cores in a closed system described in Fig. 2. Prior to rate measurements, cores were attached to the gas recirculation system and flushed with moistened air for 10 to 15 minutes. This was achieved using a manifold which divided the air stream into four portions, each leading to a soil core. With the on-off valve at point B open, and the quick connect at point C broken, the entire recirculation system could be flushed. Closing valve B, and reconnecting the gas line at C, isolated each core as a closed system. Acetylene was added to each core and the gas recirculated through the soil core and sample loops (D) on a gas chromatograph by means of a membrane pump. Typical flow rates were 300 $ml \cdot min^{-1}$.

Samples from two soil cores could be injected alternately into a ^{63}Ni electron capture detector for N_2O determination using the gas sampling valving descibed in Fig. 3. Two gas chromatographs were used; a Perkin Elmer 910 and a Varian 3700, equipped with a total of four detectors allowing denitrification rate determination on eight cores simultaneously. Details of gas chromatograph operation and gas separation have been reported (Kasper and Tiedje, 1980). When one column leading to a detector was in the injection mode, the other column was backflushed at twice the carrier gas flow rate to remove acetylene, freon and water which have retention times longer than N_2O. Samples were injected in 5 min intervals, hence the sampling interval for an individual core was 10 min.

N_2O accumulation in the recirculating gas was measured in the presence of 20% (v/v) acetylene. This relatively high acetylene concentration was used to insure that blockage of further N_2O reduction was complete. Linear rates of N_2O production could be determined within

Fig. 3 Valve configuration used to inject gas samples onto the column of the gas chromatograph and to direct flow to the detector or vent.

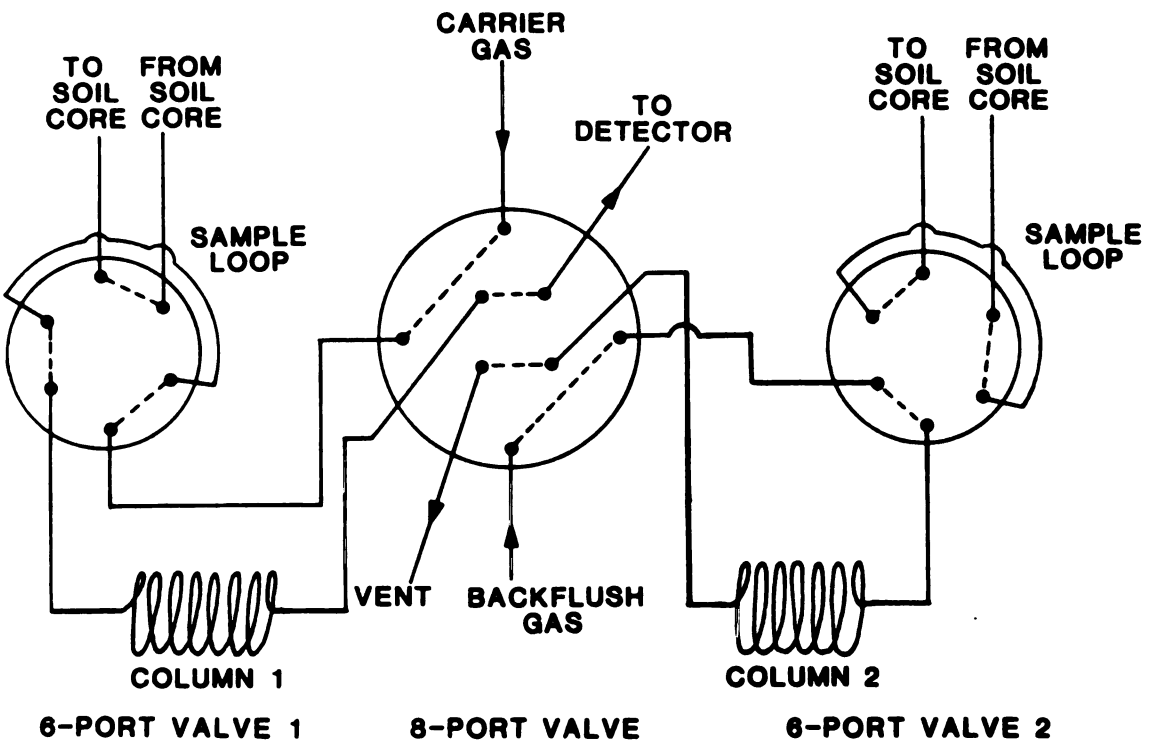
CARRIER GAS
TO SOIL CORE
FROM SOIL CORE
TO DETECTOR
TO SOIL CORE
FROM SOIL CORE
SAMPLE LOOP
SAMPLE LOOP
VENT
BACKFLUSH GAS
COLUMN 1
COLUMN 2
6-PORT VALVE 1
8-PORT VALVE
6-PORT VALVE 2

Figure 3

1 to 2 hours. Rates are reported on a per gram soil basis. Addition of acetylene to the soil cores resulted in a dilution of the O_2 content of the recirculating gas from 21% to 18%. The O_2 concentration in the recirculation gas could be adjusted to match that measured at the field sites with an *in situ* gas sampling technique (Parkin and Tiedje, 1983). For the cases reported here, soils always exhibited greater than 14% O_2 in their macropores.

Mean daily denitrification rates were determined from the individual rates of soil cores collected at each sample time. We have determined that denitrification rates at these field sites follow a log normal distribution (Parkin et al., 1983). Statistics appropriate to this distribution were used to calculate mean daily rates and 95% confidence intervals from formulas according to Blais and Carlier (1968). Use of these expressions results in asymmetric confidence intervals. Total nitrogen losses from the entire sampling period were calculated by integrating the mean daily losses over the time course of each field experiment.

Laboratory Denitrification Measurements

Freshly collected field cores from Site I and Site III were used in laboratory incubations to determine the temporal response of denitrification following water input. For these experiments 2 cm of distilled water was added to the soil surface prior to incubation on the gas recirculation system in the presence of 18% O_2 and 20% acetylene. Cores which received no water additions served as controls. Concentrations of N_2O-N in the recirculating gas phase were determined at 30 to 60 min intervals for periods of up to 24 hours.

A second experiment was performed to monitor denitrification losses from a uniformly fertilized and wetted soil. Soil from Site I was

coarsely sieved (0.5 cm mesh), and adjusted to 22% moisture with a solution containing KNO_3^- to a final nitrate concentration of 30 $\mu g \cdot g^{-1}$. PVC cylinders were packed with 500 g of this soil to a bulk density of 1.5 $Mg \cdot m^{-3}$. The cylinders were covered with plastic wrap and incubated in the dark at 25° C. Denitrification rates were determined on 4 to 8 cores at each of the following times: 0, 0.5, 1, 3, 5, 8, 12, 15, 19, and 22 days.

RESULTS

Sieved cores, which had uniformly distributed water and NO_3^-, exhibited initially high denitrification rates which reached a maximum of 300 $ng\text{-}N \cdot g^{-1} \cdot day^{-1}$ at 24 hours (Fig. 4). Following this period the rates declined rapidly over a 4 day period. In laboratory incubations unamended soil cores obtained from sandy loam and clay loam soil exhibited a constant rate of denitrification in the presence of a recirculating atmosphere of 18% oxygen for up to 20 hours (Fig. 5, dark squares). When water equivalent to a 2 cm rainfall was added to these soils at zero time, the rate of denitrification increased according to a temporal pattern characteristic of that soil. The denitrification rate was initially constant for 1 to 3 hours and 8 to 12 hours in the sandy loam and clay loam soils, respectively, followed by a period of increasing N_2O production. A second linear increase in N_2O, usually at least 10 times higher than the prewetted rate occurred within 5 hours in the sandy loam and within 14 hours in the clay loam soil.

Field denitrification rates were determined using short term (1 to 2 hour) incubations of replicate cores removed from the field at various times following soil wetting by irrigation or rainfall (Fig. 6). A similar temporal pattern to the laboratory incubations was observed. Denitrification rates generally returned to prewetting levels within 12 hours at the site with sandy loam soils and within 60 hours at the site with clay loam soils. In general a increase in the denitrification rate could be observed in both soils following at least a 1 cm water input.

Shown in Table 1 are the mean denitrification rate and 95%

Fig. 4 Mean denitrification rate and 95% CI of replicate soil cores packed with sieved clay loam soil which had been adjusted to 22 percent gravimetric moisture content at day 0.

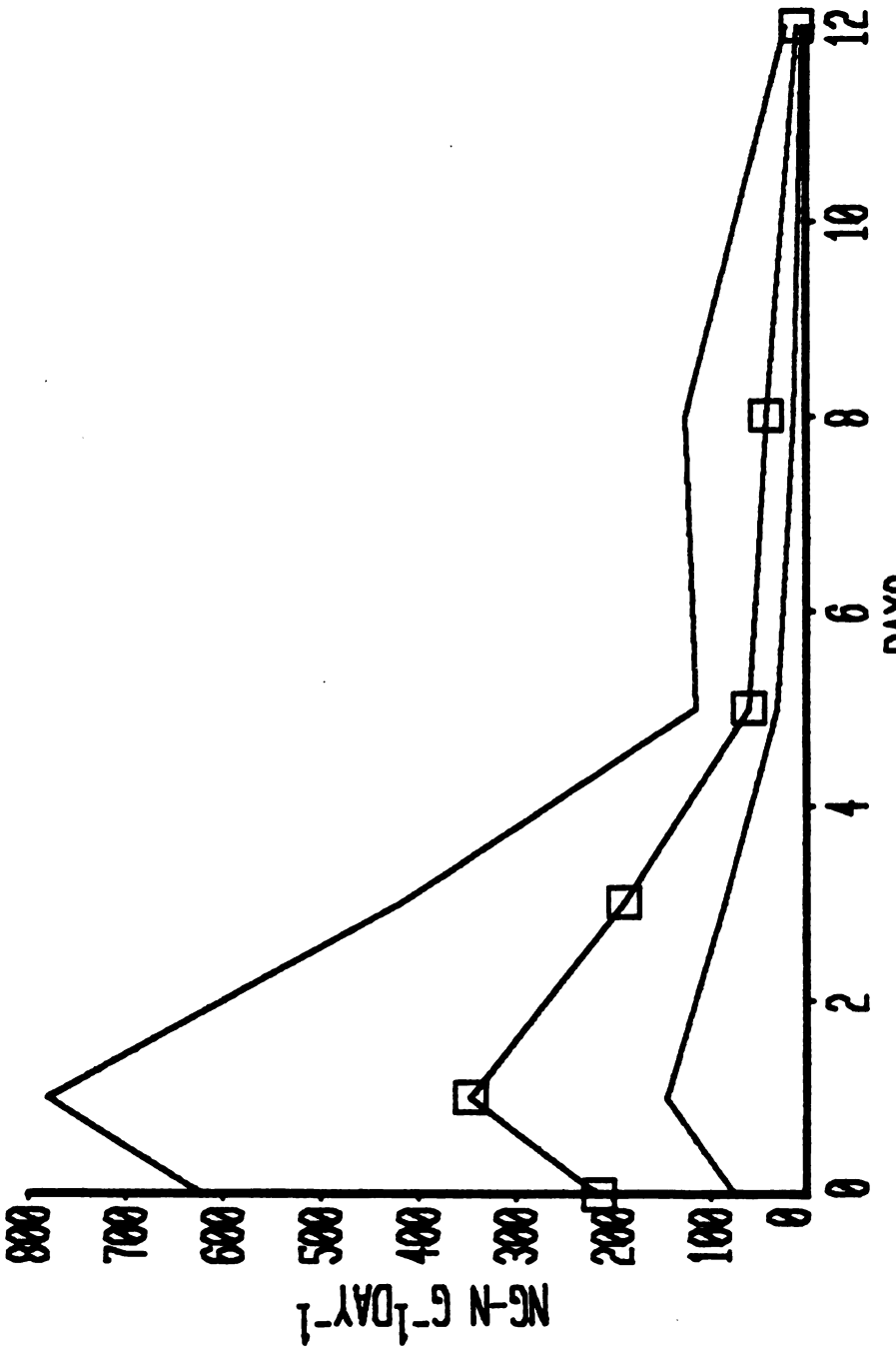

Figure 4

Fig. 5 Denitrification response of soil cores collected from the clay loam (squares) and sandy loam (triangles) soils irrigated in the laboratory with 2 cm of water at zero time. Control cores , as illustrated by the clay loam soil (dark squares) , received no water addition.

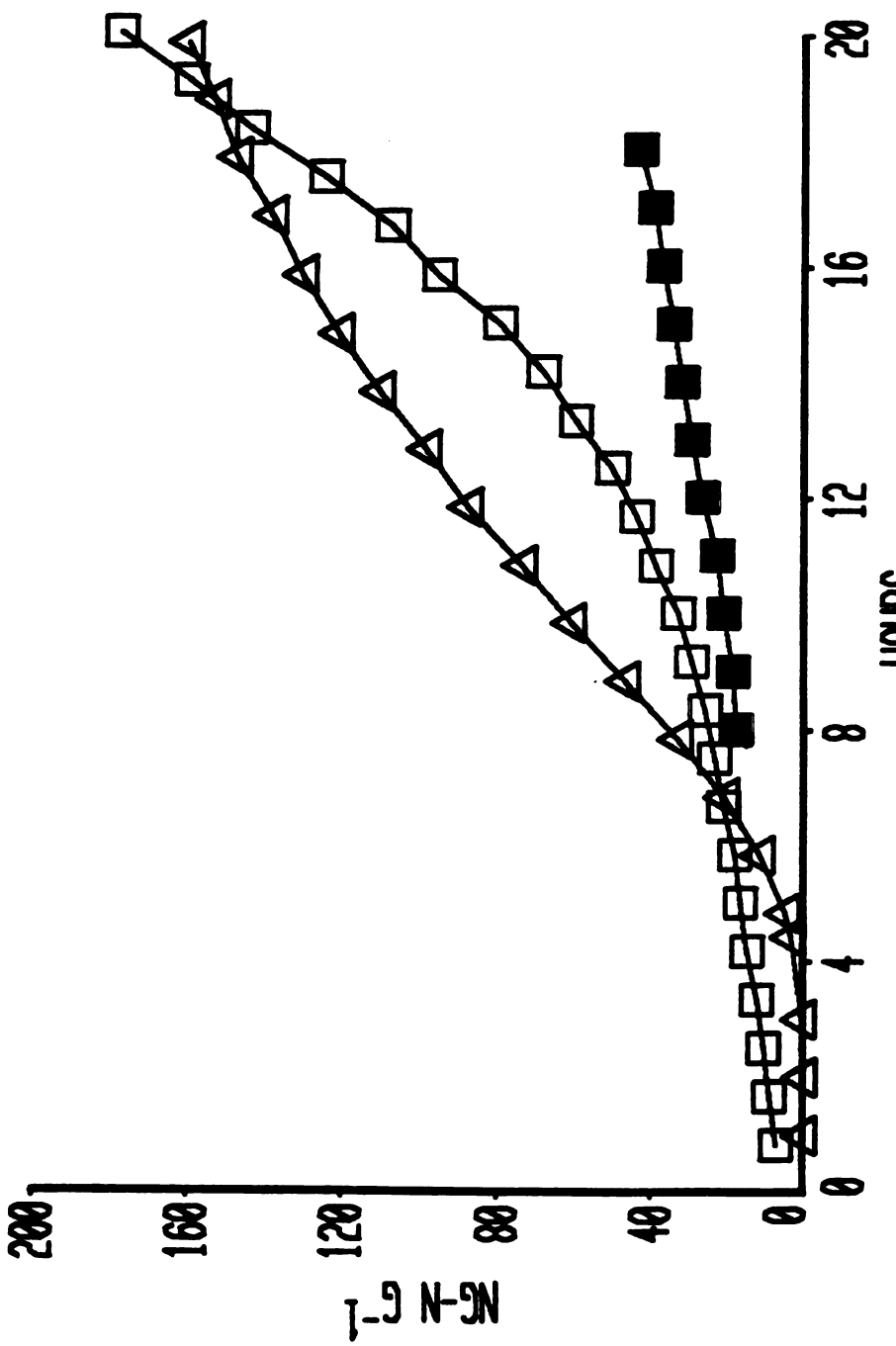

Figure 5

Fig. 6 Mean field denitrification rates of replicate soil cores collected from the clay loam (squares) and the sandy loam (triangles) soils at intervals following water inputs.

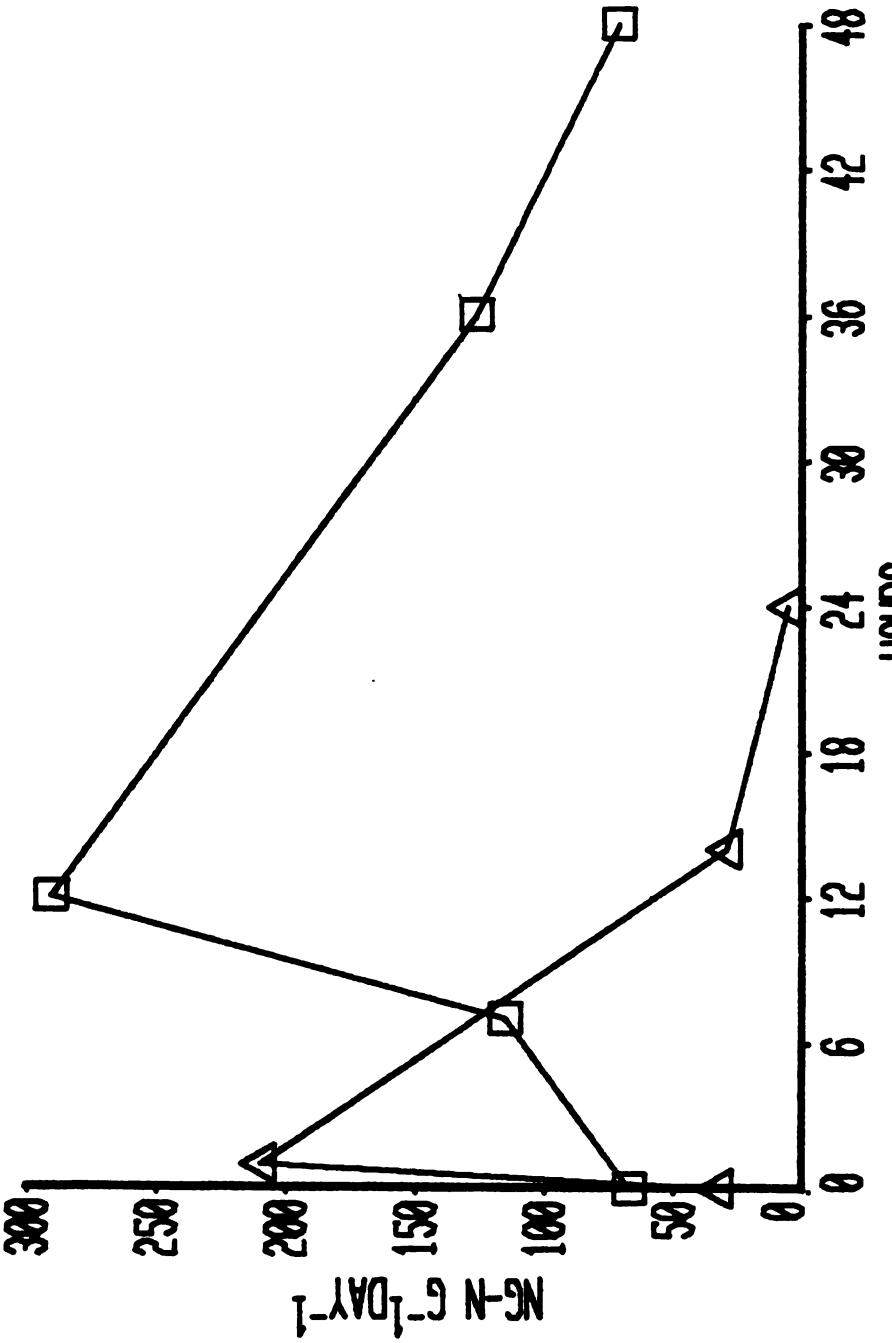

Figure 6

confidence intervals prior to and following a 7 cm and 2 cm water input to the sandy loam and clay loam soils, respectively. The mean denitrification rate increased by a factor of ten to 209 and 383 ng-N g^{-1} day^{-1}, respectively, following soil wetting when compared with prewetted levels. The increased denitrification rate following rainfall shown in Table 1 corresponded to air filled porosities of 37 to 38%. The highest denitrification rate observed at Site III was 300 ng-N g^{-1} day^{-1} and occurred following a 4 cm rainfall.

During the initial 27 days of the first field trial (Fig. 7) between 4 to 12 cores were taken almost daily. The sampling schedule was then revised to better, measure the transient temporal denitrifcation response following rainfall and to obtain increased numbers of samples to obtain better estimates of the mean rate. Following the new schedule 12 to 56 cores were collected every 3 to 4 days during dry periods, with sampling increased to every 6 to 12 hours immediately following rainfall or irrigation. This approach is illustrated (Fig. 8) with data obtained at Site II. The mean rate declined slowly following fertilization as the soil dried then increased rapidly following a 4 cm rainfall at day 7. The denitrification rate was only slightly stimulated after a second heavy rainfall (8.6 cm) at day 13, and was not increased at all by two subsequent 2 cm rainfalls. The lack of response was apparently not due to depleted soil NO_3^- since an average of 5 ppm was present in the top 30 cm of this soils on day 19. A similar sampling schedule was also followed at the sandy loam site (Site III). The four irrigation inputs were all greater than 2 cm and caused increased denitrification rates in all cases (Fig. 9).

The mean cumulative N-loss obtained by integrating the area under the daily N-loss curves shown in Figs. 7 and 9 is summarized in Table 2.

Table 1. Denitrification rates and air filled porosity prior to and following irrigation.+

Soil	Time after irrigation	Air filled porosity	Gravimetric moisture	Denitrification rate	
				Mean	95% C.I.
	-(hr)-	-(%)-	-(%)-	-(ng-N·g^{-1}·day^{-1})-	
Sandy loam	#	48	12	32	14-77
	1	37	15	209	64-680
	12	42	14	29	20-43
Clay loam	#	53	18	20	10-45
	12	37	24	383	233-615
	60	43	21	35	15-70

+ Sprinkler irrigation used to apply 7 cm and 2 cm of water to the sandy loam and clay loam, respectively.

Collected immediately prior to irrigation.

Fig. 7 Mean daily denitrification rates from a clay loam soil receiving the the indicated water inputs. Samples were collected during May-July 1981.

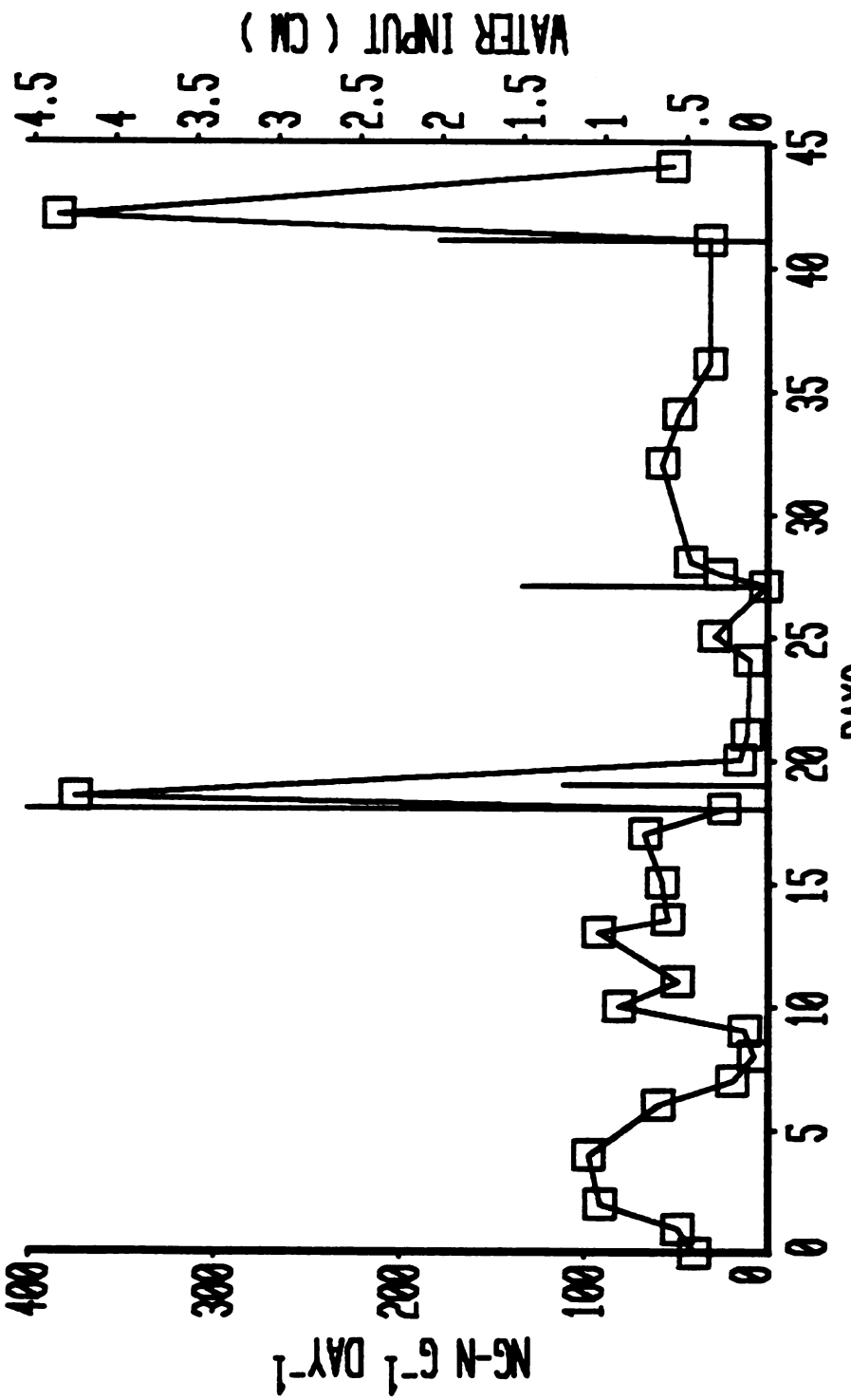
WATER INPUT (CM)
4.5
4
3.5
3
2.5
2
1.5
1
.5
0
MG-N G⁻¹ DAY⁻¹
400
300
200
100
0
0
5
10
15
20
25
30
35
40
45
DAYS

Figure 7

Fig. 8 Mean daily denitrification rates, including 95% confidence intervals of the mean,from a clay loam soil receiving the indicated rainfall. Samples were collected during September 1981.

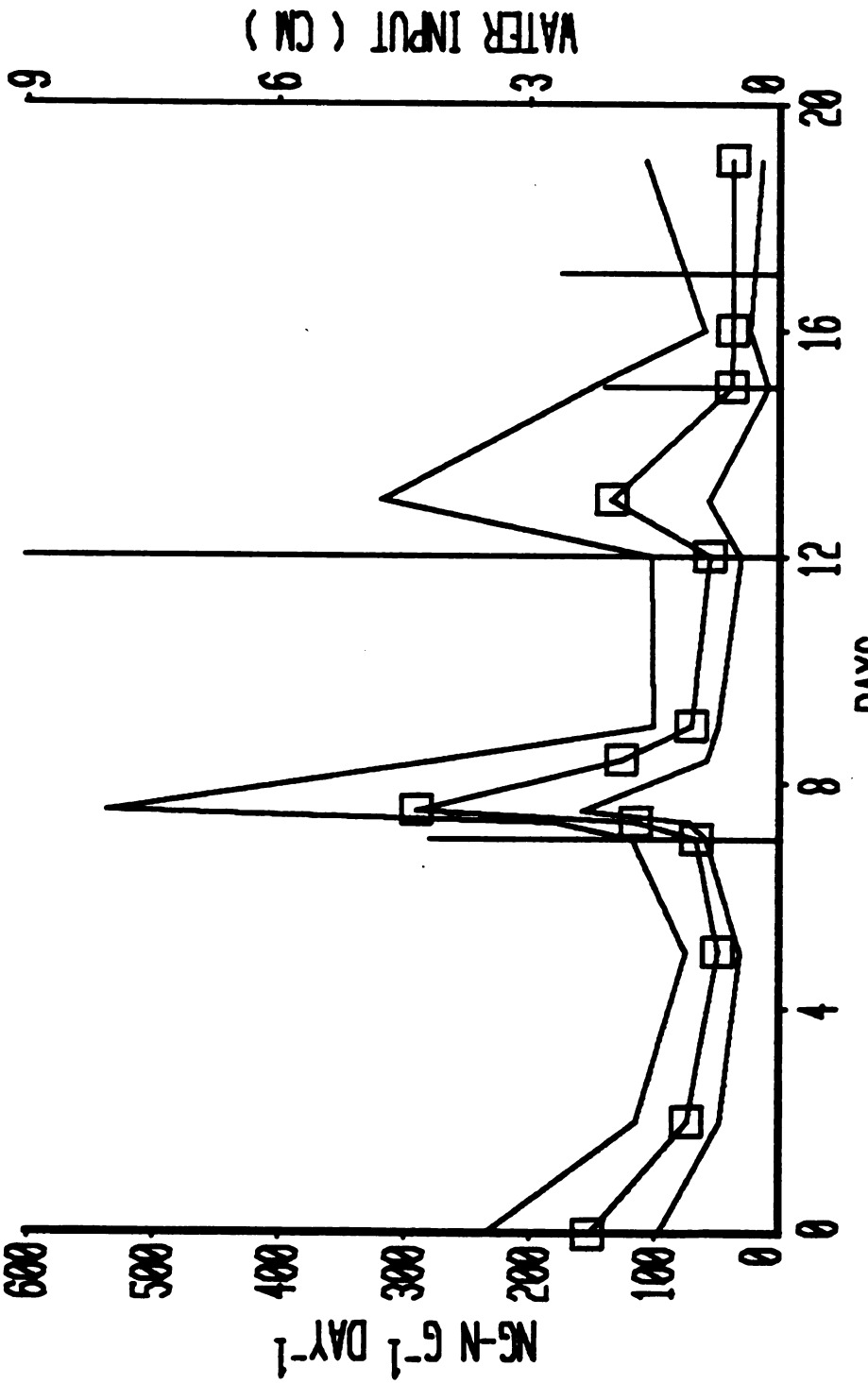
WATER INPUT (CM)
9
6
3
0
MG-N G-1 DAY-1
600
500
400
300
200
100
0
0
4
8
12
16
20
DAYS

Figure 8

Fig. 9 Mean daily denitrification rates from a sandy loam soil receiving the indicated water inputs. Samples were collected during May-July 1982.

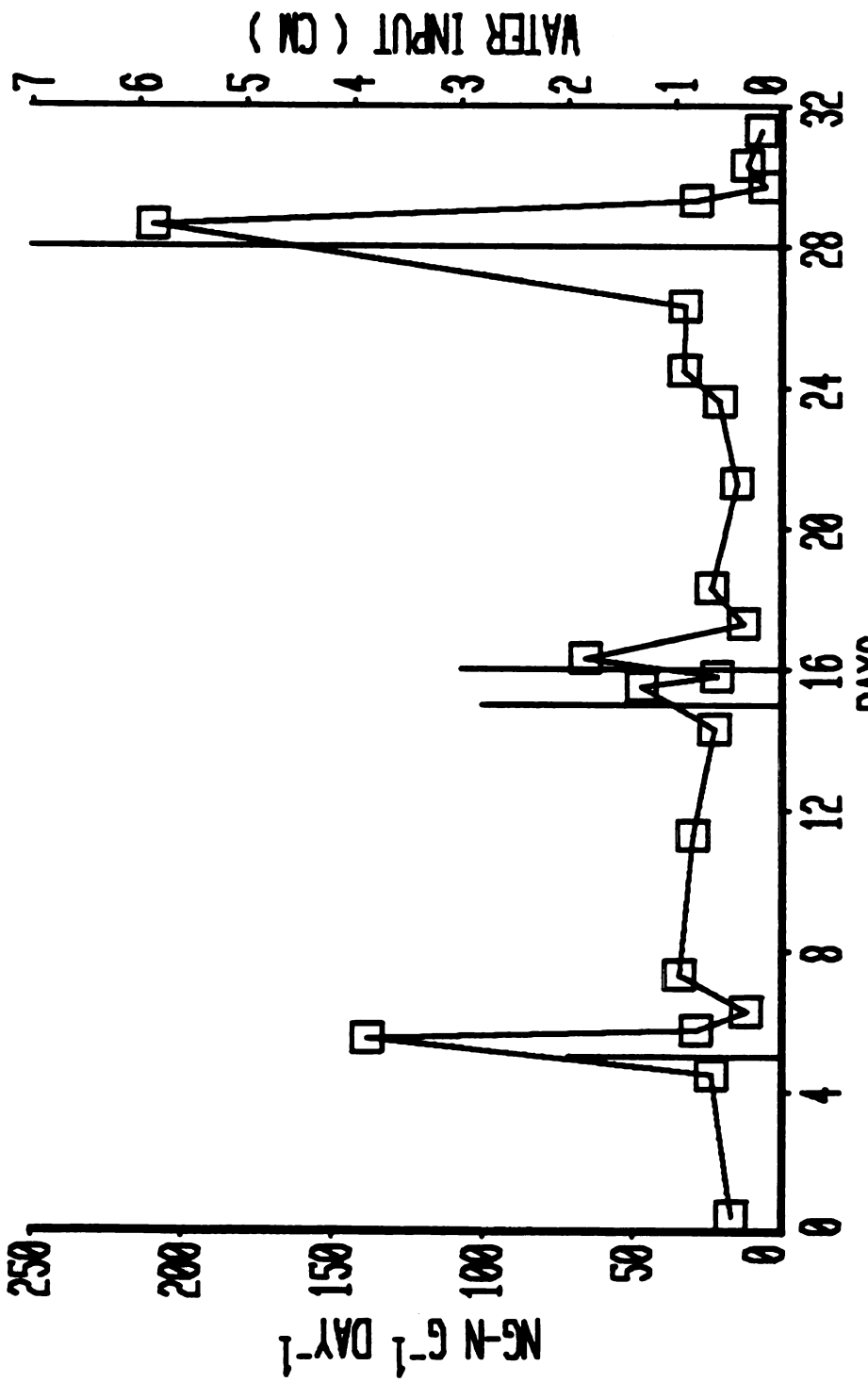
WATER INPUT (CM)
0
1
2
3
4
5
6
7
DAYS
0
4
8
12
16
20
24
28
32
MG-N G⁻¹ DAY⁻¹
0
50
100
150
200
250

Figure 9

Table 2. Comparison of cumulative nitrogen losses from two field sites as influenced by rainfall or irrigation.

Soil	Total N-loss	N-loss after water input+	Percent of total loss due to water	Total water input
	- ($\mu g\text{-}N \cdot g^{-1}$) -		- (%) -	- (cm) -
Sandy loam	1.1	0.6	55	17(4)#
Clay loam	3.2	1.2	38	9(4)

+ based on N-losses during 48 hours following rainfall.

number of water inputs of greater than 1 cm.

Also shown are the cumulative amounts of water added to the two sites. The nitrogen loss from the clay loam soil was double that of the sandy loam although the sandy loam received almost twice the water input. This difference reflects the temporal duration of the period of highest N-loss following water input in this heavy and light textured soil.

DISCUSSION

A characteristic temporal response of denitrification to periods soil wetting was observed in both laboratory and field studies. Increased denitrification rates after wetting did not occur until after a characteristic lag time, which depended on soil texture. Smith and Tiedje (1979) reported that up to 8 hours was required before increased N_2O production was observed in a sandy loam soil held at 94% of water saturation in the presence of an aerobic atmosphere. Rice and Smith (1983) observed that increased N_2O flux occurred within a few hours from soil cores of a no-tilled silt loam soil, but observed a delay of up to 12 hours before an increased flux was observed in the same soil that had been conventionally tilled. They attributed this difference to increased denitrifying enzyme content in the no-tilled soil which maintained a higher moisture content. Water infiltration, as well as gas exchange at the core surface, may have been facilitated in the no-till samples due to increased surface cracking with this tillage treatment (C.W. Rice, personal communication). Ryden et al. (1979) and Ryden and Lund (1980) noted in field measurements of denitrification, that increases in N_2O flux did not occur for 12 hours after soil wetting and that as long as 30 to 60 hours was required before peak flux occurred. A portion of the observed time dependence before increased N-flux was observed in these previous field studies was likely due to the time required for N_2O to diffuse through the soil matrix to the soil surface. Since the gas recirculation system used in our studies facilitates an equilibrium with the circulating pore space gas (Parkin

et al., 1983a), it is likely that the lag period we observed reflects the time necessary to establish the conditions for denitrification following soil wetting.

A major effect of increased soil moisture is thought to be a decreased rate of oxygen supply leading to the establishment of anaerobic zones or microsites where denitrification can occur (Smith, 1980). The time required to establish these anaerobic sites will in turn be affected by the rate of water infiltration through the soil profile. Gilliam et al. (1978) have previously suggested that any factor that decreases water flow in a soil is likely to increase denitrification. The sandy loam soil used in this study is non-aggregated and does not hold water readily, while the clay loam soil has good structure and holds more water at any given moisture potential. Water infiltration rates at similar sites to those used in this study have previously been determined to be 30 cm $hour^{-1}$ for the sandy loam, and 5 cm $hour^{-1}$ for the clay loam soil (A.E. Erickson, personal communication). It is likely that a sufficient diffusion barrier t_o oxygen allowing significant denitrification in the sandy loam soil occurs only for a brief periods following soil wetting as water percolates through the soil macropores. Slower water infiltration would require a longer period of time for anaerobiosis to be established in the clay loam soil, which may explain the longer lag period prior to a rate increase in this soil. Increased water retention in the intra-aggregate pore spaces of the clay loam soil may also explain the longer duration of increased denitrification rates.

When the soil was relatively dry, rate estimates could be extrapolated over a 2 to 3 day period providing that a sufficient number of cores were evaluated. It was necessary to sample more frequently

around rainfall or irrigation events to obtain good rate estimates during these transient periods of increased nitrogen loss. As others have suggested, strategies that rely on infrequent sampling will likely not provide good estimates of nitrogen loss from soils because of these rapid transient responses (Svensson et al., 1981). Thus, taking more cores but less frequently and concentrating on the moisture events allows calculation of the confidence intervals of the mean daily rates, information that is not available from previous studies.

Expressed on an aereal basis assuming an active depth of 20 cm, N-losses from the two sites correspond to 6.1 and 11.6 kg $N \cdot ha^{-1} \cdot mo^{-1}$. The peak mean rate observed at the clay loam site was 1.9 kg $N \cdot ha^{-1} \cdot day^{-1}$ and was observed following a 4 cm rain. The mean moisture content at the time of the highest nitrogen loss corresponded to an air filled porosity of 30%. The highest mean rate observed on the sandy loam was 1.1 kg $N \cdot ha^{-1} \cdot day^{-1}$ following a 7 cm irrigation, corresponding to an air filled porosity of 37%. The magnitude of the peak denitrification rate measured at both sites was similar, but the response at the sandy loam site occurred after higher moisture additions and was of shorter duration. Ryden et al. (1979) reported that the N_2O fluxes from a coarse textured sandy loam soil low in native organic matter were an order of magnitude lower than those observed from fine textured loam sites. Previous work by Aulakh (1982) also showed increased denitrification rates at two clay loam sites when AFP was reduced fell below 40% following rainfall. He observed the highest rates at a third fallow clay loam site where AFP average 30% during early summer. Losses at these sites were lower than those reported in this study averaging 0.5 to 1.5 kg $N \cdot ha^{-1} \cdot mo^{-1}$. Ryden et al. (1979) observed higher denitrification losses of between 7.9 and 19.5 kg

$N \cdot ha^{-1} \cdot mo^{-1}$ with maximum losses occurring with an AFP of between 8 and 15%.

After the soil dried, AFP averaged 48% and 54% on the sandy loam and clay loam soil, respectively. During this time a relatively constant low level of N_2O emission occurred in both soils. Since 20% C_2H_2 was recirculated through the cores during the denitrification assay, this N_2O emission cannot be due to nitrification (Walter et al., 1979). Some of the N_2O might result from nitrate respirers, which have been reported to produce N_2O from nitrate and to outnumber denitrifiers in soil (Smith and Zimmerman, 1981; Bleakley and Tiedje, 1982). However, only very low levels of N_2O (between 0.5 and 1.0 ng $N \cdot g^{-1} \cdot day^{-1}$) could be determined from selected cores incubated in the absence of C_2H_2, suggesting that a majority of the N_2O measured resulted from denitrification.

When irrigated corn is grown on soils similar to those used here the soil AFP seldom exceeds 33-36% (Vitosh et al, 1980; Darlington, 1983) and can decrease to 30% following irrigation. These low AFP values correspond with peak denitrification rates observed in this study. These high moisture contents coupled with N fertilization would suggest that a sustained nitrogen loss due to denitrification would occur under these conditions. However under these sustained wetting conditions, other factors may also limit nitrogen losses. The lack of a denitrification response at the fall study site following a heavy rain emphasizes that anaerobiosis alone is not sufficient for denitrification to proceed.

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CHAPTER II

INTERACTIVE CONTROL OF SOIL DENITRIFICATION RATES BY OXYGEN AND MOISTURE

Studies with pure cultures of various denitrifying bacteria have shown that the oxygen concentration must be very low or non-detectable for synthesis of denitrifying reductases to occur (Firestone,1982; Knowles,1982). Exposure to oxygen subsequent to synthesis causes enzyme inactivation; whether by direct enzyme inhibition, or action as a competitive electron acceptor is not yet clear. Various studies have previously established that oxygen is an important regulator of soil denitrification (Pilot and Patrick,1972; Stefanson,1973; Gilliam,1978). Control of denitrification by oxygen has not yet been clearly quantitated however, such that relationships predicting the denitrification rate under various oxygen conditions can be established. This task is made difficult by the three phase nature of soil, and further complicated by temporal and spatial variability of denitrification. The soil atmosphere in a water non-saturated soil is usually aerobic, thus anaerobic sites with potential for denitrification to occur only due to restricted oxygen diffusion through the soil and the respiratory oxygen consumption by the soil biota. Mathematical expressions to predict the size of these anaerobic zones have been developed (Currie, 1961; Greenwood, 1961) but are not experimentally validated. The size of anaerobic zones should be determined in part by soil texture, moisture, and respiratory demand. These factors may also have other important effects, for example soil moisture may determine distribution of soluble carbon and nitrate, important substrates for denitrifying bacteria. It is clear that to realistically measure oxygen

control of denitrification the physical structure of the soil must be considered.

We have previously described an acetylene soil core system to measure denitrification rates in relatively undisturbed samples (Parkin et al.,1983). A primary advantage of this method is that denitrification rates can be determined in the presence of an aerobic atmosphere with the extent of soil anaerobiosis being controlled by the structure of the soil core. Rates can be determined rapidly in 1 to 2 hours, allowing transient temporal responses to be followed. Finally, the soil moisture content and pore space oxygen concentration of soil cores can be experimentally varied, allowing control of denitrification by these factors to be investigated . In this paper we report the use of this method to investigate the interactive control of the soil denitrification rate by oxygen and moisture.

MATERIALS AND METHODS

Soil cores for these studies were collected during the summers of 1981 and 1982 from a clay loam and sandy loam field site as described in the previous chapter. Cores were returned to the laboratory and stored at 4° C prior to use. The soil moisture content was adjusted by adding various amounts of water to the surface of the core, and incubating the cores at 25°C for 12 hours prior to denitrification rate determinations. We have previously determined that maximum denitrification rates occur within 12 hours of core wetting in these soils (Chapter I). Control cores received no water additions. Between four to eight cores were measured for each moisture content.

Denitrification rates were determined in the presence of 20% acetylene. Using a gas recirculation technique coupled with a gas chromatograph equipped with an electron capture detector, concentrations of O_2 and N_2O could be periodically determined (Parkin et al., 1983a). In some experiments soil respiration rates were determined by CO_2 accumulation. The recirculating gas was 18% O_2 for aerobic rate determinations and argon for anaerobic rate determinations. Some cores were sieved after denitrification rate measurements and 50 grams of the soil mixed with 50 ml H_2O in a slurry to determine an additional anaerobic rate in a manner similar to the technique described by Smith and Tiedje (1979).

Denitrification rates were also determined over a range of oxygen concentrations on individual soil cores. Subsequent to an aerobic rate determination, the cores were flushed with argon for 10 to 15 min to

remove all oxygen from the recircuation system. The oxygen concentration in the soil pore spaces was then adjusted by injecting varying amounts of oxygen or air. Acetylene was again added and a denitrification rate determined over a 1 to 2 hour period. The procedure was repeated at several oxygen concentrations and terminated with an anaerobic rate determination. Rates at each oxygen concentration were normalized as a percentage of the anaerobic rate (%AR) to facilitate comparisons among cores.

The following empirical hyperbolic expression was used to describe the data obtained from the cores incubated over a range of pore space oxygen concentrations (PSO):

$$\%AR = 100P/(PSO)+P$$

Best fits to the experimental data, and estimates of the parameter P, were obtained using a random search parameter estimation program designed to minimize the error sum of squares ("R'EVOLV", courtesy Dr. J. A. Robinson). Estimates of P were used to solve this expression for the pore space oxygen concentration necessary to achieve 10% of the anaerobic rate for each core. The data were also fit to a model that predicts soil anaerobic volume as a function of PSO (Smith, 1980). Use of the model requires values of the intra-aggregate diffusion coefficient (D_a), the soil respiration rate (Q), and an estimate of the log mean aggregate diameter. Values of Q were experimentally determined by measuring rates of CO_2 production during the aerobic rate determinations. A log mean aggregate diameter of 1 mm and 0.5 mm was selected for the clay loam and sandy loam, respectively from values discussed by Gardner (1952). Simulations were generated by varying D_a from $1x10^{-6}$ to $5x10^{-4}$ $cm^2 \cdot s^{-1}$. Values of the diffusion coefficient

giving the best fit to the experimental data were visually interpolated from this family of curves as described by Parkin and Tiedje (1983).

RESULTS

The effect of simulated rainfall on the denitrification rate of soil cores collected from a clay loam site are shown in Table 1. Increased denitrification rates were observed with increasing water additions both when the rates were determined in the presence of 18% oxygen and under anaerobic conditions. The anaerobic rate represents the maximum rate for an individual core since all oxygen control has been removed. Anaerobic rates in this soil ranged from 1 to 9 $\mu g\text{-}N \cdot g^{-1} \cdot day^{-1}$. Expressing the aerobic rate as a percentage of the anaerobic rate (%AR) has been shown to reduce variability for comparisons by normalizing data among cores (Parkin and Tiedje, 1983). Aerobic rates determined from soils collected from dry soils ranged between 1 to 20% of their respective anaerobic rate. In a similar experiment cores collected subsequent to a 2 cm irrigation exhibited an increased %AR when wet to a similar moisture content as cores not doubly wetted. When these cores were wetted to 63% of saturation, up to 60% of the potential anaerobic rate was observed in the presence of 18% oxygen.

Increased anaerobic rates with increasing soil moisture may be caused either by increased denitrifying enzyme content in the wetter soil, or perhaps by redistribution of soluble carbon or nitrate to additional active sites within the soil. This possibility was investigated by measuring denitrification rates on anaerobic soil slurries, prepared after first determining aerobic and anaerobic rates on cores (Table 2). The slurry assay removes all diffusive limitations and can be used as a measure denitrifying enzyme content of the sample

Table 1. The effect of water addition on denitrification rates in a clay loam soil.

	Water input	Moisture content	Aerobic denitrification rate	Anaerobic denitrification rate	%AR
	-(cm)-	-(%)-	-(ng-N·g^{-1}·day^{-1})-		
Prior to irrigation	0	18.6	29 (103)#	1920 (44)	1.4 (64)
	1	22.1	67 (72)	3457 (63)	1.8 (63)
	2	26.2	382 (102)	3656 (75)	8.6 (77)
After 2 cm irrigation	0	19.7	444 (95)	3384 (31)	11.1 (80)
	1	26.9	604 (100)	4636 (49)	16.3 (117)
	2	30.6	3826 (20)	6622 (24)	58.1 (4)

coefficient of variation

(Smith and Tiedje, 1979). In the clay loam soil, anaerobic rates increased in wetted soils, however no increase in denitrifying enzyme content was detected. The slurry rates were ten times higher than the respective anaerobic rate, indicating that substrate redistribution could be important in increasing the observed rates in this soil. Slurry and anaerobic rates were similar in a largely non-aggregated sandy loam soil, suggesting that significantly more substrate would not be available at additional active sites in this soil after wetting.

Increasing the oxygen concentration from anaerobiosis to 1% and 5% in the pore space of cores from the clay loam soil reduced the denitrification rate to 17.5% and 13.5% of the former anaerobic rate (Fig.1) The lower rate was established in less than 10 min, demonstrating the rapid control of denitrification even at relatively low concentrations of oxygen. The effect of oxygen concentration on denitrification rates in cores of the sandy loam and clay loam soil was further examined at pore space oxygen contents from 18% to complete anaerobiosis. The resulting data of %AR vs. pore space oxygen could be fit to a hyperbolic expression (Figs. 2,3). Wetter cores showed a higher %AR than drier cores at comparable pore space oxygen concentrations and exhibited the characteristically rapid hyperbolic increase in rate at higher pore space oxygen concentrations. These data were used to obtain the pore space oxygen concentration necessary to achieve 10% of the anaerobic rate at a given air filled porosity (Fig.4). Air filled porosity was used instead of percent moisture to more easily compare soils of different texture. The oxygen concentration for 10% AR decreased with decreasing air filled porosity in both soils; however at any given air filled porosity, much lower oxygen concentrations were

Table 2. Effect of water addition on denitrification rates in a clay loam and sandy loam soil.

	Water input	Moisture content	Denitrification rate: Aerobic core	Denitrification rate: Anaerobic core	Denitrification rate: Anaerobic slurry
	-(cm)-	-(%)-	-(ng-N·g^{-1}·day^{-1})-		
Clay loam	0	19.6	117 (112)#	860 (50)	20,439 (31)
	1	23.2	362 (120)	1519 (50)	18,835 (17)
	2	25.5	684 (73)	2175 (40)	19,550 (23)
Sandy loam	0	12.4	5 (74)	694 (125)	630 (98)
	1	14.7	16 (96)	707 (62)	1031 (97)
	2	17.2	31 (93)	1967 (93)	1007 (80)

coefficient of variation

required in the coarser textured sandy loam soil compared with the clay loam. At air filled porosities less than 30%, the clay loam soil exhibited greater than 10% of the anaerobic rate in the presence of 18% oxygen.

Parkin and Tiedje (1983) first observed the hyperbolic relationship between %AR and pore space oxygen concentration, and suggested the data resembled simulations by K.A. Smith (1980) on the percent anaerobic volume versus pore space oxygen concentration. They used Smith's model to fit experimental data for soils at a single moisture content, by assuming both a soil respiration rate and value for the intra-aggregate diffusion coefficient. In the present work, we have data over a range of soil moisture contents, and have also experimentally measured the soil respiration rate. We were interested to see how values of the intra-aggregate diffusion coefficient which provided the best fit to our data would vary with soil moisture content. The oxygen diffusion coefficient is known to vary over four orders of magnitude from its value in air to 10^{-6} $cm^2 \cdot s^{-1}$ in water (Greenwood, 1961). Experimental measurement of this parameter in soil are few, but are of primary importance in describing soil anaerobiosis and predicting denitrification rates. Examples of the fits obtained are shown in Fig. 5, and the data summarized in Table 3. The soil respiration rate generally increased with increasing soil moisture in the sandy loam soil and was of similar magnitude in the clay loam soil regardless of soil moisture . The diffusion coefficient providing the best fit to the data ranged from 8 x 10^{-5} to 3 x 10^{-6} $cm^2 \cdot s^{-1}$ in the clay loam soil. Estimates were generally higher in the sandy loam soil ranging from 3 x 10^{-4} to 3 x 10^{-5} $cm^2 \cdot s^{-1}$. We cannot yet state that this model provides

true estimates of this parameter, however the trends are reasonable and provide a useful conceptual framework for further investigation of oxygen control of soil denitrification.

Fig. 1. Effect of oxygen additions on the anaerobic denitrification rate of a clay loam soil. Oxygen added to final concentration of 5% and 1% at 50 minutes (squares) and 60 minutes (circles), respectively.

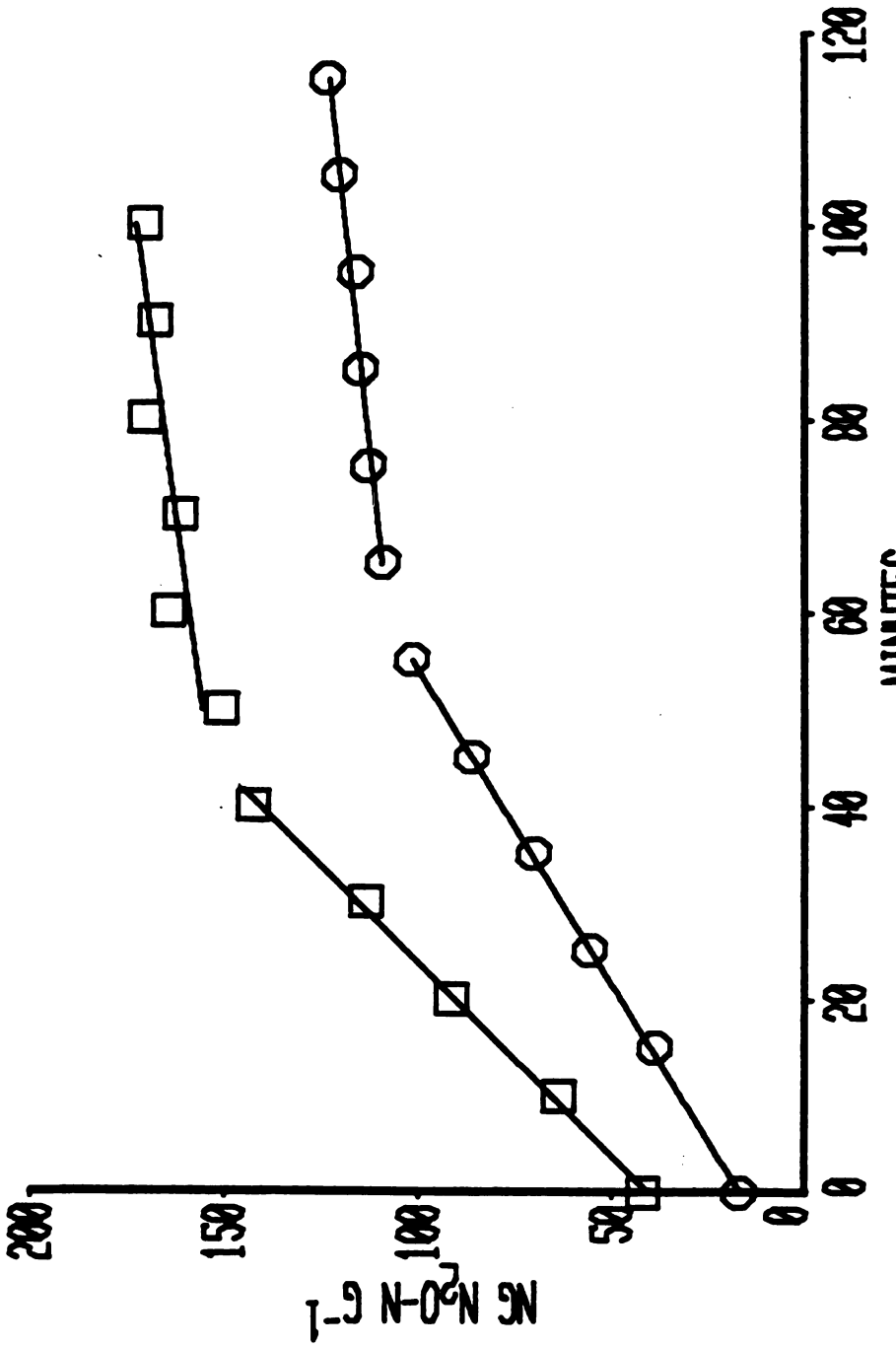

Figure 1

Fig. 2. Aerobic denitrification rates in a clay loam soil at various oxygen concentrations. Rates are expressed as a percentage of the anaerobic rate. The moisture content was 24%(squares) and 17% (circles). The solid line was obtained by fitting the data to a hyperbolic equation.

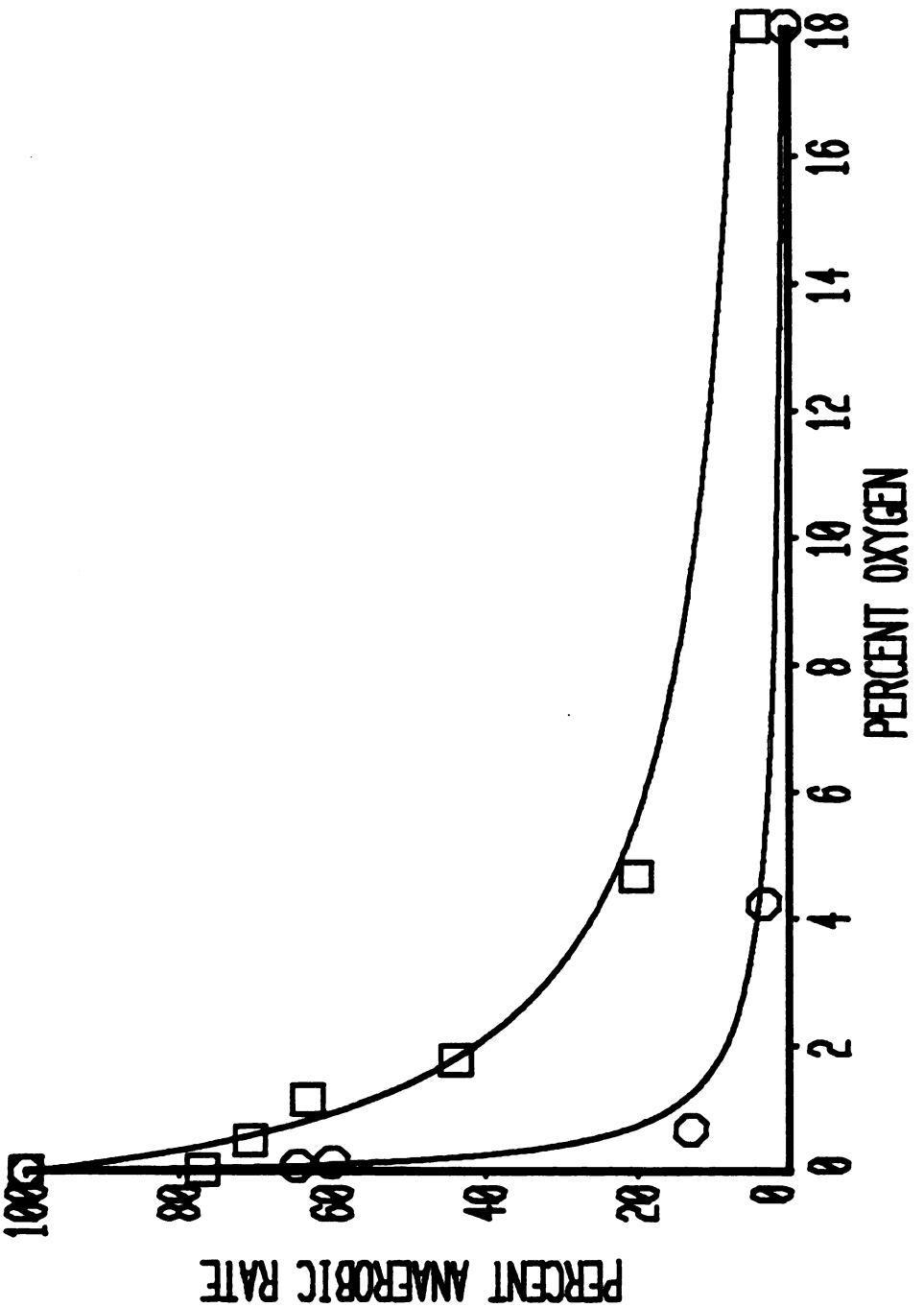
PERCENT ANAEROBIC RATE
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
PERCENT OXYGEN

Figure 2

Fig. 3. Percentage of the anaerobic rate at varying pore space oxygen concentrations in a sandy loam soil. The moisture content was 12% (squares) and 17% (circles).

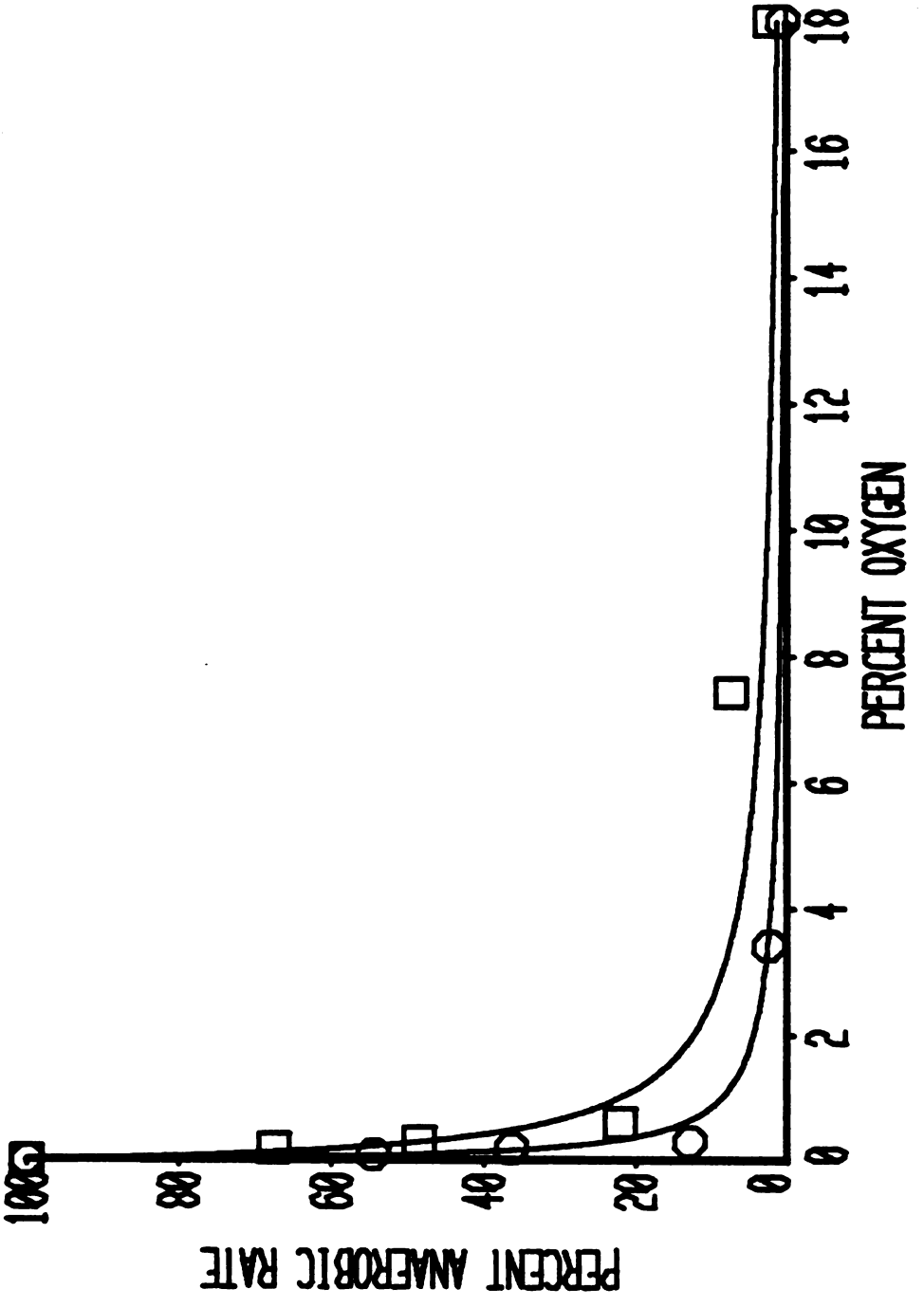
PERCENT ANAEROBIC RATE
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
PERCENT OXYGEN

Figure 3

Fig. 4. Pore space oxygen concentration necessary to achieve 10% of the anaerobic rate for cores at varying air filled porosities. Data for clay loam (squares) and sandy loam (circles).

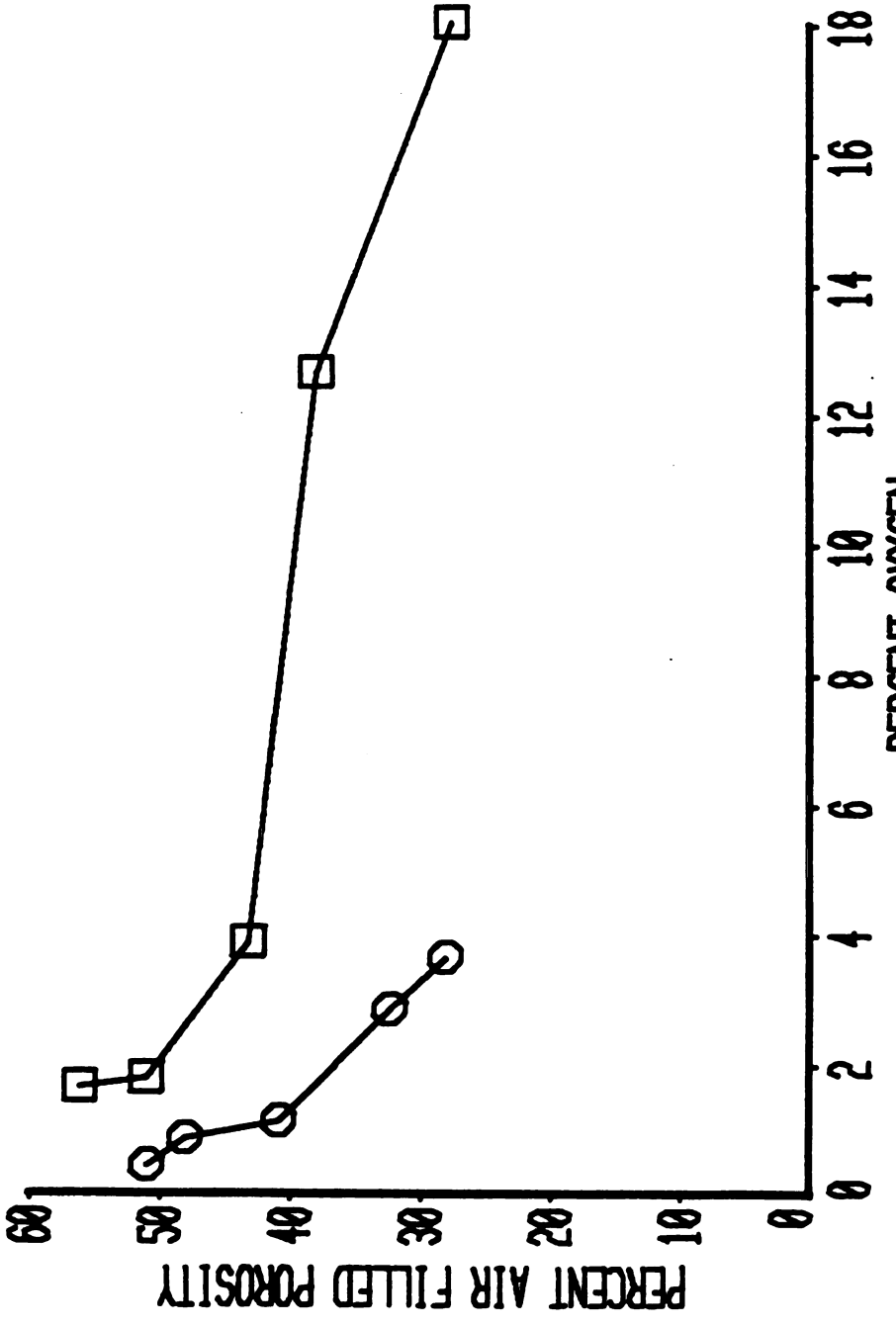
PERCENT AIR FILLED POROSITY
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
PERCENT OXYGEN

Figure 4

Fig. 5. Best fits of numerical simulations of % anaerobic volume vs. pore space oxygen concentration (symbols) to experimentally determined relationship between % anaerobic rate and pore space oxygen concentration (solid lines). Clay loam at 28% moisture fit using D_a of 3.0 x $10^{-6} cm^2 \cdot s^{-1}$ (squares). Sandy loam at 17% moisture fit using D_a of 3 x 10^{-5} $cm^2 \cdot s^{-1}$ (circles)

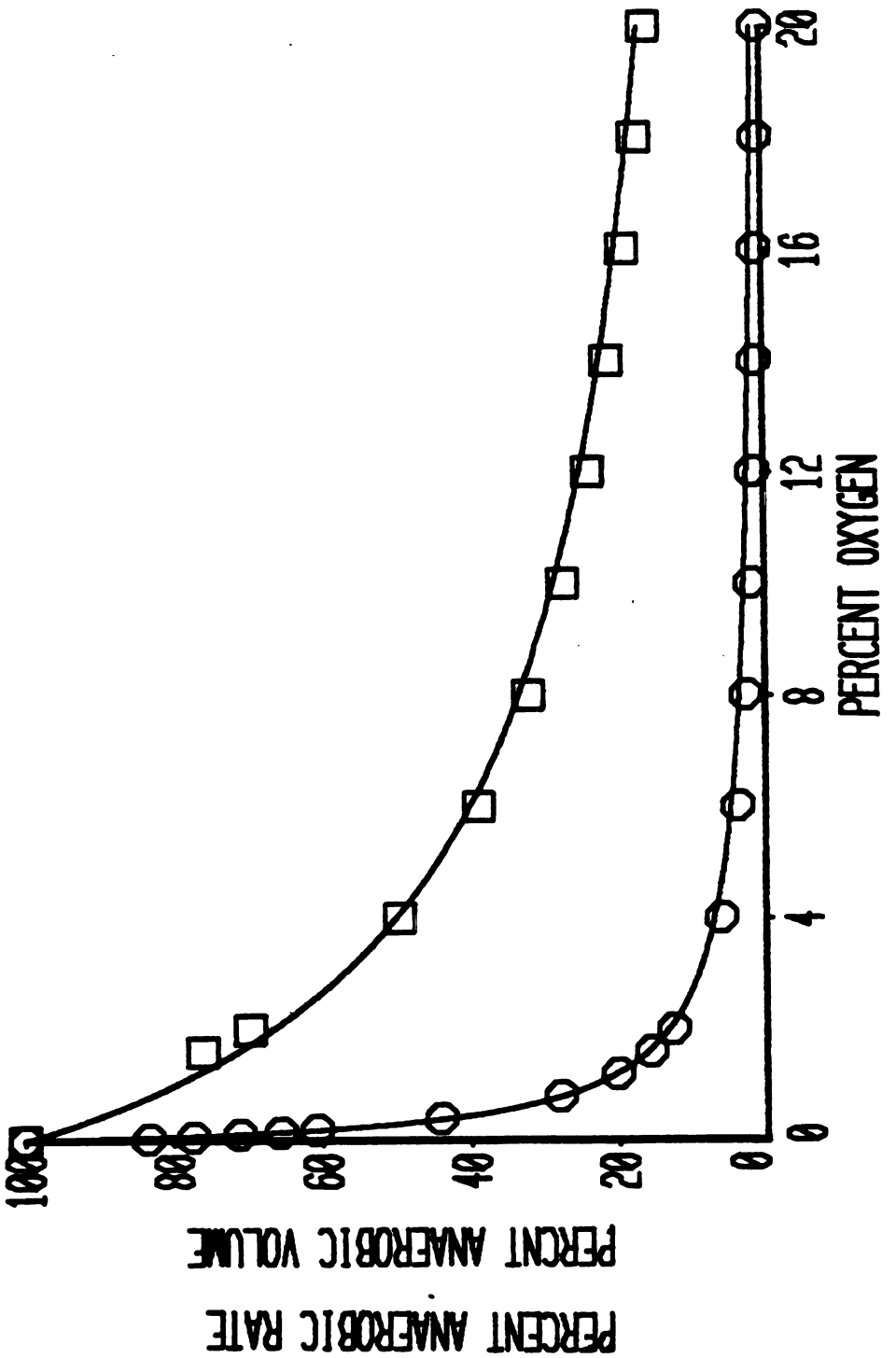
PERCENT ANAEROBIC RATE
PERCENT ANAEROBIC VOLUME
100
80
60
40
20
0
0
4
8
12
16
20
PERCENT OXYGEN

Figure 5

Table 3. Respiration rates and estimated intra-aggregate diffusion coefficient for a clay loam and sandy loam soil.

	Moisture content	Respiration rate	Intra-aggregate oxygen diffusion coefficient
	-(%)-	$-(cm^3 \cdot cm^{-3} \cdot s^{-1})-$ x 10^7	$-(cm^2 \cdot s^{-1})-$ x 10^6
Clay loam	17	11.0	80
	24	10.9	10
	28	6.4	3
Sandy loam	12	3.8	300
	14	6.7	80
	17	24.9	30

DISCUSSION

When evaluating his model, Smith determined that lowering the intra-aggregate diffusion coefficient 10-fold dramatically increased the soil anaerobic volume. Our estimates of D_a, obtained by fitting experimental data to this model from soils displaying a range of moisture contents and with known respiration rates, varied over an order of magnitude in both soils. Using Smith's model with our data assumes that there is a direct correlation between %AR and the percent anaerobic soil volume. The striking similarity in the relationship of %AR and anaerobic volume as a function of oxygen concentration suggests similarity in the physical process effecting these two parameters. Our data indicate that the soil denitrification rate is inversly related to the level of pore space oxygen present, and that this relationship is further effected by the soil moisture content. According to Smith, decreasing the pore space oxygen concentration or increasing the soil moisture content would be expected to increase the soil anaerobic volume, due to decreased oxygen diffusion.

The diffusion coefficients estimated from the model were lower in the clay loam soil than in the sandy loam soil. This is consistent with the relationship presented in Fig 5., which demonstrates that at the same air filled porosity the clay loam soil could have a greater pore space oxygen concentration and still achieve the same relative denitrification rate when compared with the sandy loam soil. We chose to construct this relationship for 10% of the anaerobic rate, because this is often the peak level of denitrification N-loss from these soils

following rainfall or irrigation (Chapter I). In these previous studies, soil pore space oxygen concentrations of less than 15% were seldom measured (Parkin et al., 1983b). High denitrification losses can occur from the clay loam soil at these oxygen concentrations if the air filled porosity is less than 30%. Very low air filled porosities would be required in the sandy loam to achieve similar losses. The field response in the sandy loam soil lasted only a few hours, presumably while the soil macropores were largely saturated with water. It will be important to construct oxygen moisture relationships for soils of additional textures to obtain predictive relationships of expected denitrification responses.

The estimated diffusion coefficients are affected by the log mean aggregate diameter chosen for the simulations. We used a value of 1 mm with the clay loam soil, which is within the range discussed for fine textured soils by Gardner (1956). Doubling this value would increase the estimated values for D_a by 2 to 3 times. The value of 0.5 mm used with the sandy loam soil may be too large. This soil is only weakly aggregated, containing 70% sand particles. Simulations using a lower mean diameter predicts that the anaerobic volume of this soil would be extremely small, except at very low diffusion coefficients and low pore space oxygen concentrations. According to Smith's formulation increased soil respiration rates can have an equal effect to decreased oxygen diffusion in determining anaerobic volume. The rates we measured were similar in magnitude to those reported by Greenwood (1975), but varied over a narrow range. We measured respiration rates on a whole core basis, and it is possible that localized areas of much higher activity exist within the core. This could cause localized anaerobiosis not

predictable by the existing model, and may further explain the occurance of denitrification rates observed in the coarser textured soil.

Previous workers have shown that nitrogen losses from soil depend on the oxygen concentration of the incubation, as well as the soil moisture content. Pilot and Patrick (1972) attempted to obtain quantitative relationships of factors controlling N-loss. They concluded that higher nitrogen losses were correlated with fine soil texture and low air filled porosity. However, like many previous studies they worked with disturbed, sieved soils in long term incubations, missing important short term dynamics of denitrification in a structured soil. The acetylene soil core technique used in these studies has proved very versatile. We have been able to measure short term rates under conditions of natural oxygen status, as well as to experimentally vary soil anaerobiosis. Under conditions of complete anaerobiosis, it is possibe to investigate other factors that may influence denitrification rates, once oxygen control has been removed. Unlike previous workers, we could not demonstrate increased denitrifying enzyme content with increasing soil moisture content (Smith and Tiedje, 1979), although anaerobic denitrification rates increased with increasing soil moisture. We attribute this increase to substrate redistribution with increasing soil moisture content. The extent of such an increase will also depend on substrate availability. We have previously observed the denitrification response to decrease with sequential wettings in a fall sampled field site, perhaps due to exhausted supply of available carbon (Sexstone et al., 1983). The role of substrate supply to anaerobic sites of denitrification appears complex, and deserves additional experimental attention.

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CHAPTER III

DIRECT MEASUREMENTS OF OXYGEN PROFILES AND AND DENITRIFICATION RATES IN SOIL AGGREGATES

Synthesis by bacteria of denitrifying N-oxide reductases can occur in the presence of reduced oxygen concentrations, however, total anaerobiosis is required for expression of denitrifying activity (Firestone, 1982). Denitrification activity can, however, be measured in soils containing atmospheric oxygen concentrations in their macropores (Sexstone et al., 1983). Anaerobic microsites, within soil aggregates have been suggested to explain this observation (Greenwood, 1961; Currie, 1961). These microsites are thought to occur when oxygen consumption exceeds diffusive supply. The volume of these microsites has been calculated assuming radial diffusion, an average aggregate respiration rate and an estimated intra-aggregate O_2 diffusion coefficient. These calculations have been incorporated into recent mathematical models in attempts to predict soil denitrification rates (Smith, 1980; Leffelaar, 1979; McGill et al., 1981).

Soil macroaggregates can vary widely in size (1 to 100 mm in diameter) and are constructed from complex smaller assemblages of soil primary particles, humic materials, roots, as well as microbial biomass and polymers (Harris et al., 1966). A major limitation to the investigation of oxygen within aggregates is a suitable technique with the necessary spatial resolution to measure concentrations over very small distances. Oxygen electrodes provide one approach to this problem. Oxygen can be electrochemically reduced on a gold or platinum electrode surface

that is negatively charged at 0.6 - 0.8 volts relative to a Ag/AgCl reference electrode. The current produced is proportional to the amount of oxygen reduced and ideally is proportional to the oxygen concentration in the medium. However, if the gold or platinum cathode is large and is in direct contact with the medium, then significant oxygen consumption will occur and the measured current will reflect only the rate of oxygen supply to the electrode.

Various amperometric techniques have been used to measure soil aeration. Bare platinum electrodes (Lemon and Erickson, 1952) have been used to measure oxygen flux to the electrode surface. These electrodes were initially developed to describe critical moisture values for sufficient oxygen supply to plant roots. Limitations to the theory and use of bare platinum electrodes in soil has been discussed in detail by McIntyre (1970). Membrane covered Clark-type electrodes have also been used to measure oxygen partial pressure in soil (Clark, 1953). These electrodes were used initially by Willey and Tanner (1963) but were dependent on the ability of the medium to maintain an oxygen supply to the cathode tip. Subsequent improvements by Enoch & Falkenflug (1968) produced electrodes that were equally sensitive in a gaseous or liquid phase, but exhibited a very slow response time (10 min to 2 hours) to changes in oxygen concentration. Fluhler et al. (1976) used this type of electrode to monitor oxygen concentrations throughout a soil profile in response to water ponding. Characteristic of all of the preceding devices is their relatively large size, such that the

measurement obtained reflects an average value over many potentially different aeration sites.

One previous study by Greenwood & Goodman (1967) used an oxygen electrode (Naylor & Evans, 1960) with characteristics sufficient to obtain oxygen profiles in 10 mm diameter molded spherical aggregates saturated in 0.1 N KCl. Oxygen consumption by the cathode was minimized by applying the polarization voltage for only 0.3 seconds at 10 sec intervals. Complete anaerobiosis was recorded within 2 mm from the surface of their aggregate constructs. The sensing tip of the electrode was 0.5 mm in diameter, which was judged by these workers to be too large. They suggested that localized disturbance and soil compaction occured at the electrode tip during insertion, resulting in steeper oxygen gradients than would be theoretically predicted.

Oxygen microelectrodes have recently been used successfully to measure oxygen concentration gradients at marine sediment-water interfaces and in photosynthetic microbial mats (Revsbech et al., 1980). The total diameter of the microelectrode tip can be made as small as 1 to 2 μm. With the use of a micromanipulator to insert the electrode, oxygen gradients can be measured in as little as 0.1 mm increments. This microelectrode has recently been modified, and is now a Clark-type electrode suitable for use in soil (Revsbech & Ward, 1983). In this study, we have used these new electrodes to directly measure the occurrence of anaerobic centers in individual soil aggregates. It was also possible to obtain values for the oxygen diffusion coefficient and to calculate the

oxygen consumption rates of aggregates. The occurrence of anaerobic microsites was compared with denitrification rates measured in individual soil aggregates.

MATERIALS AND METHODS

Soil Aggregates

Aggregates obtained from two Iowa soils: a Muscatine silty clay loam obtained from an agricultural field under continuous cultivation for 50 years, and and Ackmore silt loam collected from a nearby uncultivated native prairie. The soil was collected moist in the fall, crumbled and roughly spherical aggregates ranging from 6 to 20 mm in diameter were selected. Aggregates were placed in a large closed jar in direct contact with water saturated tissue paper. Aggregates were turned periodically, and allowed to wet for 2 to 3 days prior to their use. The resulting average moisture content was 43% on a dry weight basis.

Oxygen microelectrode

Oxygen was measured using microelectrodes constructed as described by Revsbech and Ward (1983). The electrode contains an inner, gold tipped, glass coated, platinum cathode with a sensing tip diameter of 1 to 3 μm (Fig. 1). The cathode is contained within an outer glass casing, the space between the casing and the cathode being filled with a chloride containing electrolyte (Fig. 1) . A AgCl coated silver wire immersed in the electrolyte serves as the reference. The outer casing tip contains a silicone rubber membrane which is extremely permeable to oxygen. The electrode was made more rugged for use in soil by making the distal 8 to 12 mm of the shaft 0.1 mm thick. The tip itself was

Figure 1. Diagram and dimensions of the oxygen microelectrode cathode (A) and tip (B).

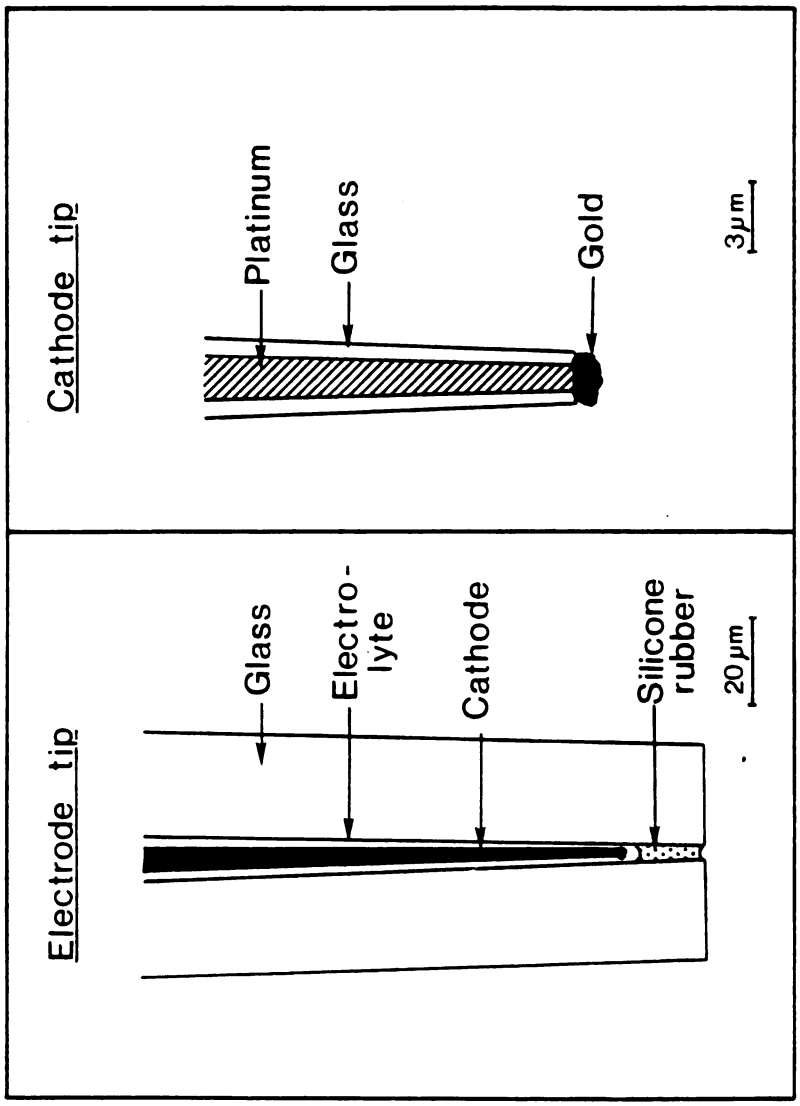
Cathode tip
Platinum
Glass
Gold
3µm
Electrode tip
Glass
Electro-
lyte
Cathode
Silicone
rubber
20µm

Figure 1

30 to 50 μm in diameter with a 2 to 3 um silicone-filled hole to allow oxygen to reach the cathode. The electrode was both sturdy and sufficiently small so that it could be inserted to ca. 10 mm depth without causing a major disturbance in the aggregate. The electrode was operated by applying a polarization voltage of 0.75 V and reading the resultant current in the measuring circuit with a Kiethley Model 480 picoampeter connected to a strip chart recorder. The 90% response of these electrodes was generally less than 0.5 sec and was linear from anaerobiosis to 100% oxygen. A two point calibration of the electrodes was made in aerated and deoxygenated water. Electrodes exhibited drift of < 1% per hour.

Oxygen Measurements

Aggregates were placed on a hollow, perforated, elevated stand in a plexiglass chamber through which a known gas mixture could be passed (Fig. 2). The perforated stand was necessary to insure that oxygen diffusion was free to occur over the entire aggregate surface. In some experiments, the aggregate was kept in contact with a water saturated tissue paper wick to insure wetness. Water saturated air, or known oxygen-argon mixtures were passed through the chamber to maintain a known external oxygen concentration at the aggregate surface. The microelectrode was lowered into the chamber and inserted into the soil aggregate by means of a micromanipulator. One to 10 oxygen profiles were obtained for each individual aggregate.

Two dimensional contour maps of the intra-aggregate oxygen concentrations were constructed from eight oxygen profiles

Figure 2. Flow through gas chamber for determining oxygen profiles in soil aggregates: A) inlet for moisturized air, argon-oxygen mixtures, N_2, or 0_2, B) perforated plastic stand to allow uniform gas dispersion over the entire aggregate surface, C) soil aggregate, D) parafilm covering with access hole for the microelectrode, E) oxygen microelectrode, F) electrode connection to picoampeter, G) micromanipulator attached the stand.

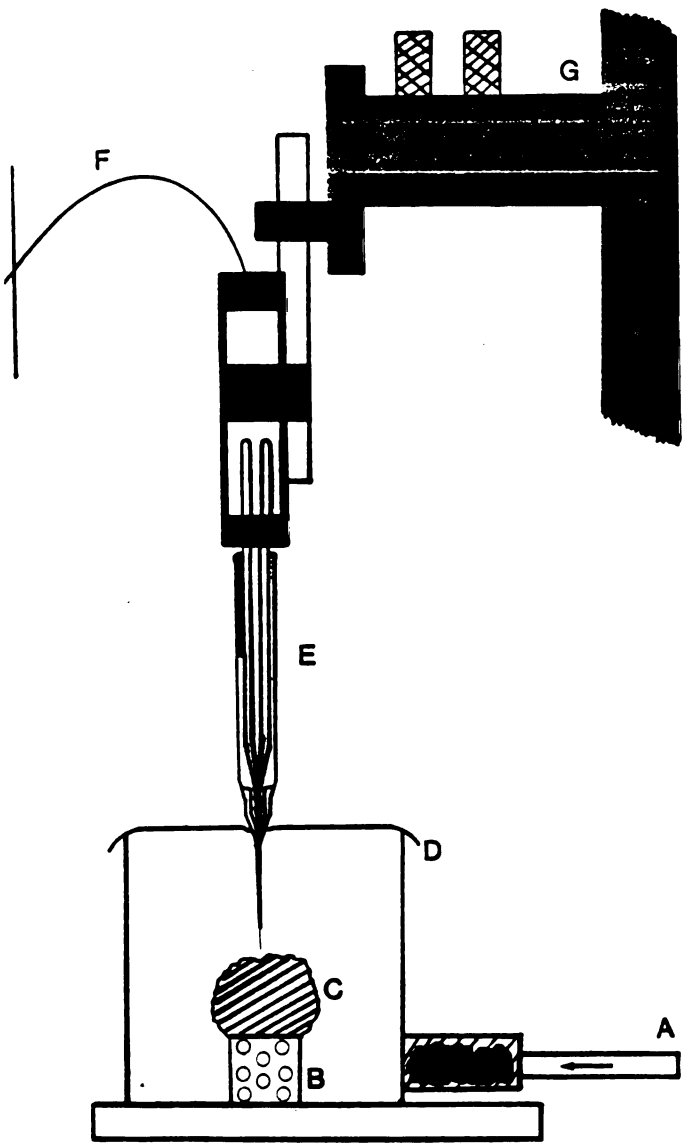

Figure 2

obtained in 45 degree increments around an aggregate circumference. The oxygen contours were calculated and plotted from this information using the Surface II Graphics System (Sampson, 1978).

Oxygen diffusion coefficient

To estimate the intra-aggregate oxygen diffusion coefficient (Da), the oxygen concentration at a fixed depth (5.35 to 1.2 mm) within the aggregate was monitored with time after the oxygen concentration outside the aggregate was abruptly changed to either water saturated 100% O_2 or 100% N_2. The increase or decrease in the oxygen concentration, respectively, was measured for 200 seconds. These determinations were made at room temperature, which was generally 23.5°C. The chamber atmosphere was then returned to moistened air and the aggregate allowed to equilibrate for 30 to 60 min before the next measurement. This procedure was repeated at three or four depths in four aggregates. The aggregates used in these experiments had radii of 8 mm or greater.

The oxygen concentration at the aggregate surface (C_o at depth x = 0) was constant throughout these experiments since the gas in the chamber was continually replaced. The integration of Fick's second law under these boundary conditions, to obtain a concentration (C) at a depth (x) and time (t) has been reported (Crank, 1956):

$$C_{(x,t)} = C_o \text{ erfc } x/2(D_at)^{1/2}$$

where erfc stands for the complementary error function. This expression was used to construct a linear error function plot of

the data as described by Duursma and Hoede (1967) from which D_a can be estimated. Simulations of the predicted oxygen concentration at a fixed depth with time were obtained by numerically integrating Fick's second law of one dimensional diffusion using the Crank-Nicholson method of finite differences (Crank, 1956).

Measurement of aggregate gases

Aggregates were enclosed in 7 ml serum bottles capped with a Balch stopper or a 15 ml plexiglass tube capped at both ends with latex rubber serum stoppers. The aggregates were placed on raised perforated stands in these containers to allow homogeneous diffusion. The headspace air was ammended with 10% C_2H_2 to measure the aggregate denitrification rate by the acetylene inhibition of N_2O reduction (Yoshinari et al., 1975). Respiration was followed by measuring CO_2 production. Periodic injections of 0.25 ml of headspace from the container were made into a Varian 3700 gas chromotograph equipped with dual ^{63}Ni ECD detectors, which were operated at 300°C and a standing current of 3 mA. The gases were separated on a Poropak Q column (1.8 m x 0.32 cm O.D) at 55°C using an argon-methane (95/5) carrier at a flow rate of 15 $ml \cdot min^{-1}$. Rates were expressed on a dry weight or total aggregate basis and were corrected for dissolved gases.

Anaerobic volume and oxygen flux measurements and calculations.

The measured anaerobic radius of an aggregate was compared with the anaerobic radius calculated according to expressions described by Smith (1980). A predicted oxygen consumption rate was estimated as the total oxygen flux (f) over the entire

aggregate surface area (S):

$$f = (S)(P)(D_a)\ (dC/dx)$$

where P is the volume percent solids (0.7) and Da the average diffusion coefficient measured for the aggregates. An average oxygen concentration gradient, dC/dx, was measured directly with the electrode from 8 to 10 concentrations measured at random at a 1 mm depth on the aggregate surface.

RESULTS

The oxygen microelectrode could be inserted into the soil aggregates in as little as 0.1 mm increments by a micromanipulator. Typical oxygen concentration profiles obtained in this way are shown in Fig. 3. Curve A shows the steep oxygen gradient that can occur over a small distance. Complete anaerobiosis occured after only 3.5 mm in this aggregate, which had a radius of 7 mm. Curve B also revealed anaerobiosis in another aggregate, however, the oxygen gradient was more gradual and less regular than the previous example. Curve C was obtained on an aggregate slightly larger than the ones for A or B, but a minimum oxygen concentration of 0.03 atm was measured at the aggregate center. The oxygen concentration increased as the electrode passed beyond the center. Curve D was obtained on a native prairie aggregate and shows surface oxygen intrusion up to the 5 mm depth within the aggregate, which can likely be attributed to root channels observed within these aggregates. Oxygen profiles were obtained on a total of 57 aggregates whose radii ranged from 3 to 12 mm. Of these, 12 aggregates exhibited at least one profile showing complete anaerobiosis. The smallest aggregate observed with an anaerobic center had a radius of 4 mm. Aggregates with radii greater than 10 mm generally exhibited at least one profile showing a zone of no oxygen, however, it was not uncommon for larger aggregates, particularly from undisturbed prairie, to have measurable oxygen concentrations throughout.

Figure 3. Examples of oxygen profiles obtained in silt loam aggregates. Aggregate radius (mm) was 7,8,9,9 for aggregates A,B,C,D, respectively. A-C were aggregates from a cultivated field; D was from an uncultivated native prairie.

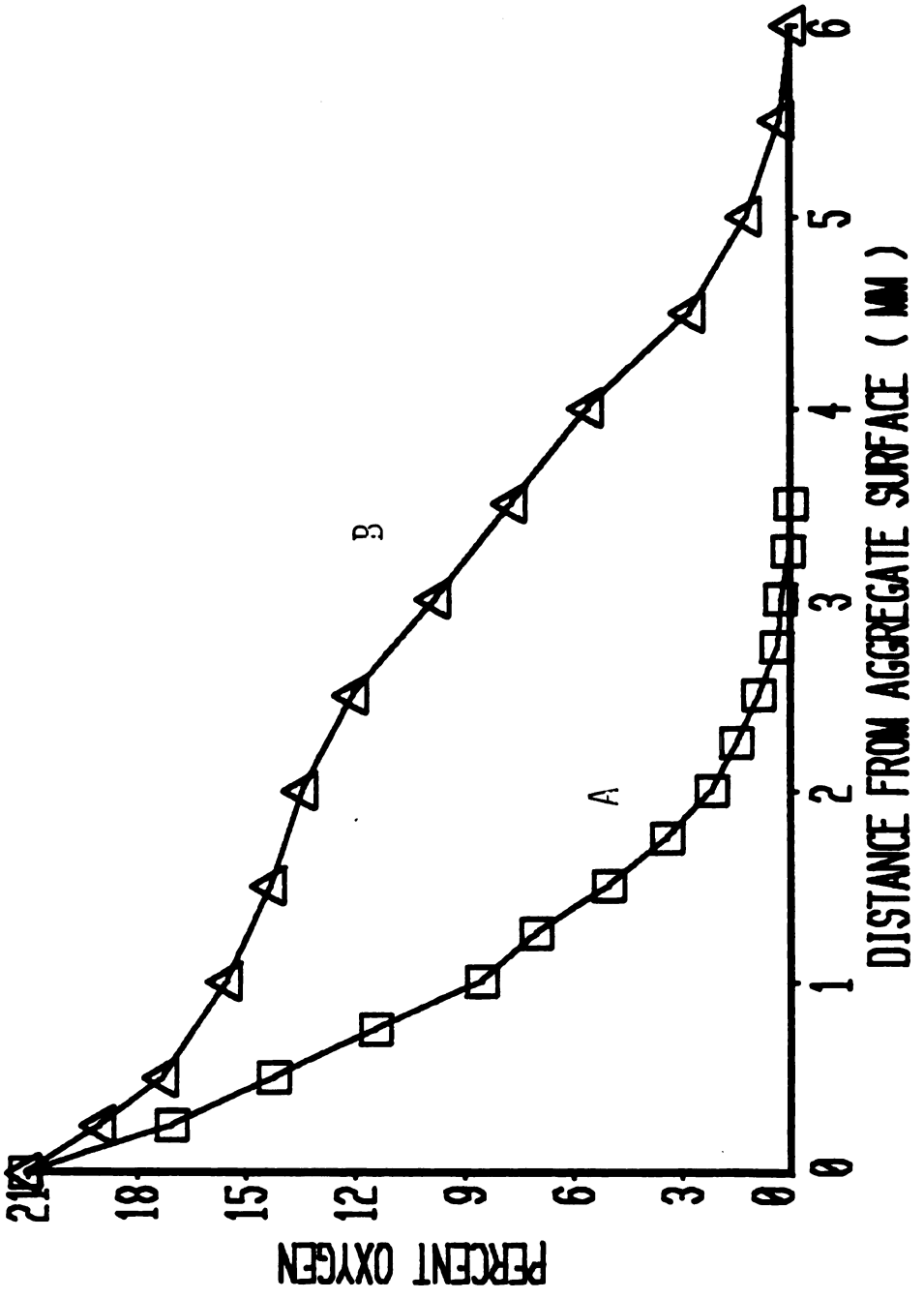
PERCENT OXYGEN
21
18
15
12
9
6
3
0
0
1
2
3
4
5
6
DISTANCE FROM AGGREGATE SURFACE (MM)
A
B

Figure 3

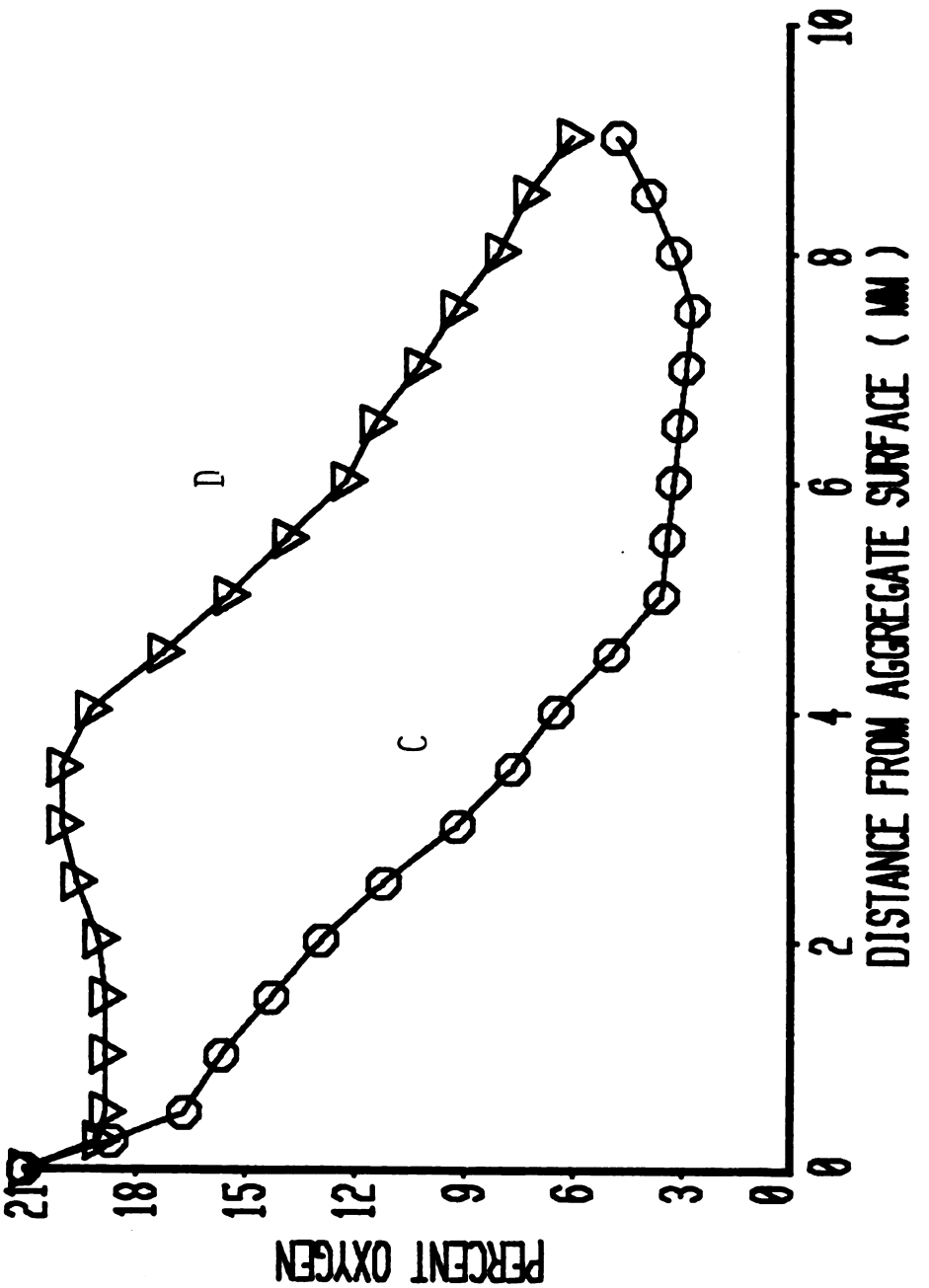

Figure 3 continued

Profiles around a circumference of selected aggregates were used to construct a contour map of intra-aggregate oxygen concentrations (Fig. 4). Aggregate A exhibited a small anaerobic zone which was skewed from the aggregate center. Aggregate B showed regular and steep oxygen gradients around the entire aggregate circumference which resulted in a large and regular anaerobic center. Prior to these measurements, this aggregate had been rolled to give the aggregate a more spherical shape. In all aggregates treated in this manner, the observed oxygen gradient increased after rolling although not all rolled aggregates exhibited anaerobic centers. Aggregate C exhibited oxygen concentrations as low as 0.01 atm but no strictly anaerobic sites were observed.

Oxygen concentrations, determined at several depths within an aggregate 200 sec after the surrounding atmosphere was rapidly changed from water saturated air to 100% oxygen, were used to determine the intra-aggregate diffusion coefficient (Fig. 5). These values were used to construct an error function plot (Fig. 6) from which a value of D_a could be determined, in this case 1.1 x 10^{-5} cm^2 s^{-1}. The closed symbols are from the experiment where the imposed gradient was 100% N_2; the same value for the diffusion coefficient was obtained. The mean oxygen diffusion coefficient determined from four aggregates was 8.5 x 10^{-6} cm^2 s^{-1}.

In addition to oxygen profiles, the denitrification and respiration rates were determined for several aggregates (Table 1). Those aggregates with a measurable denitrification rate

Figure 4. Maps of oxygen concentrations within silt-loam aggregates. A and B were aggregates from a cultivated field; C was from an uncultivated prairie.

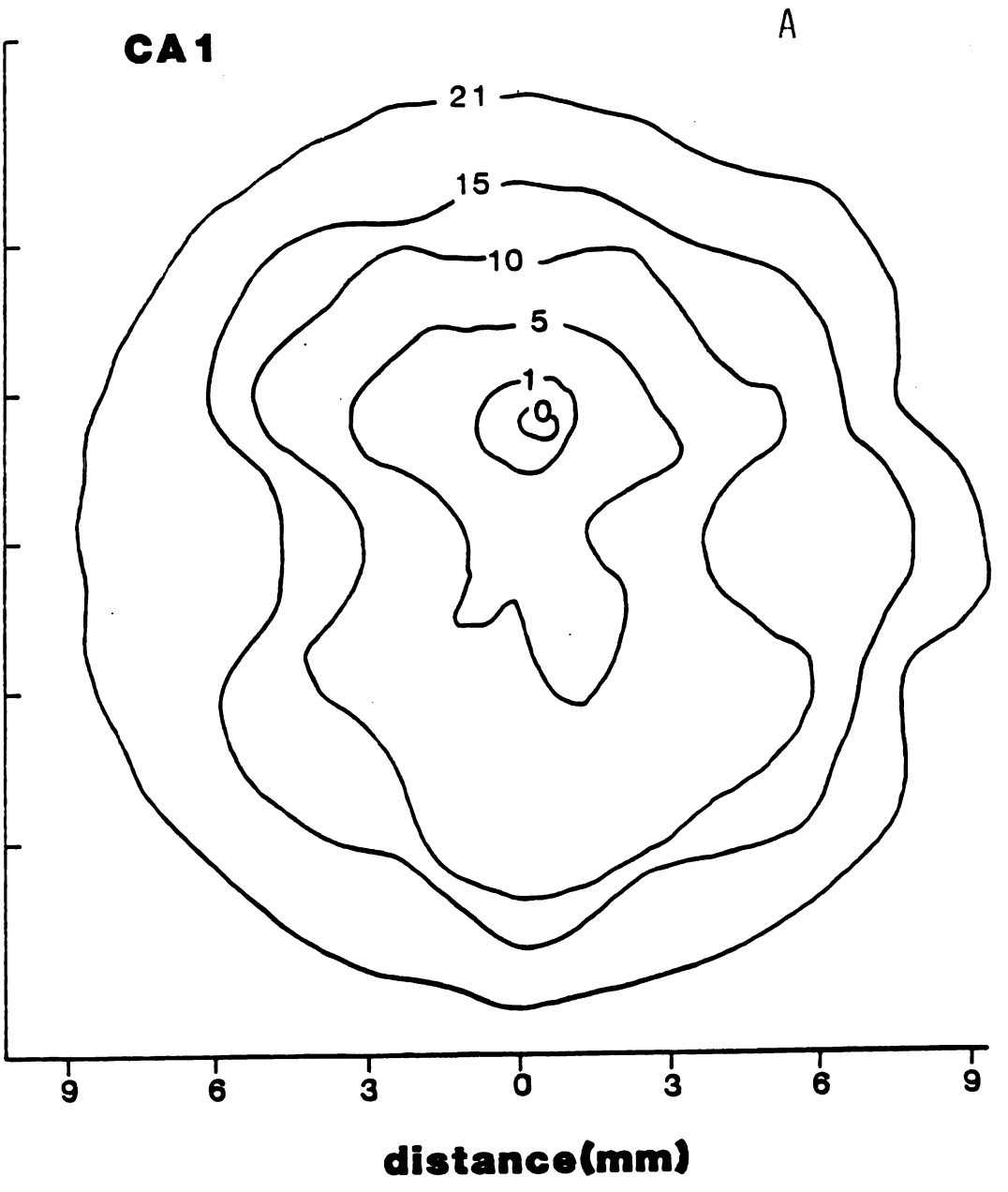

Figure 4

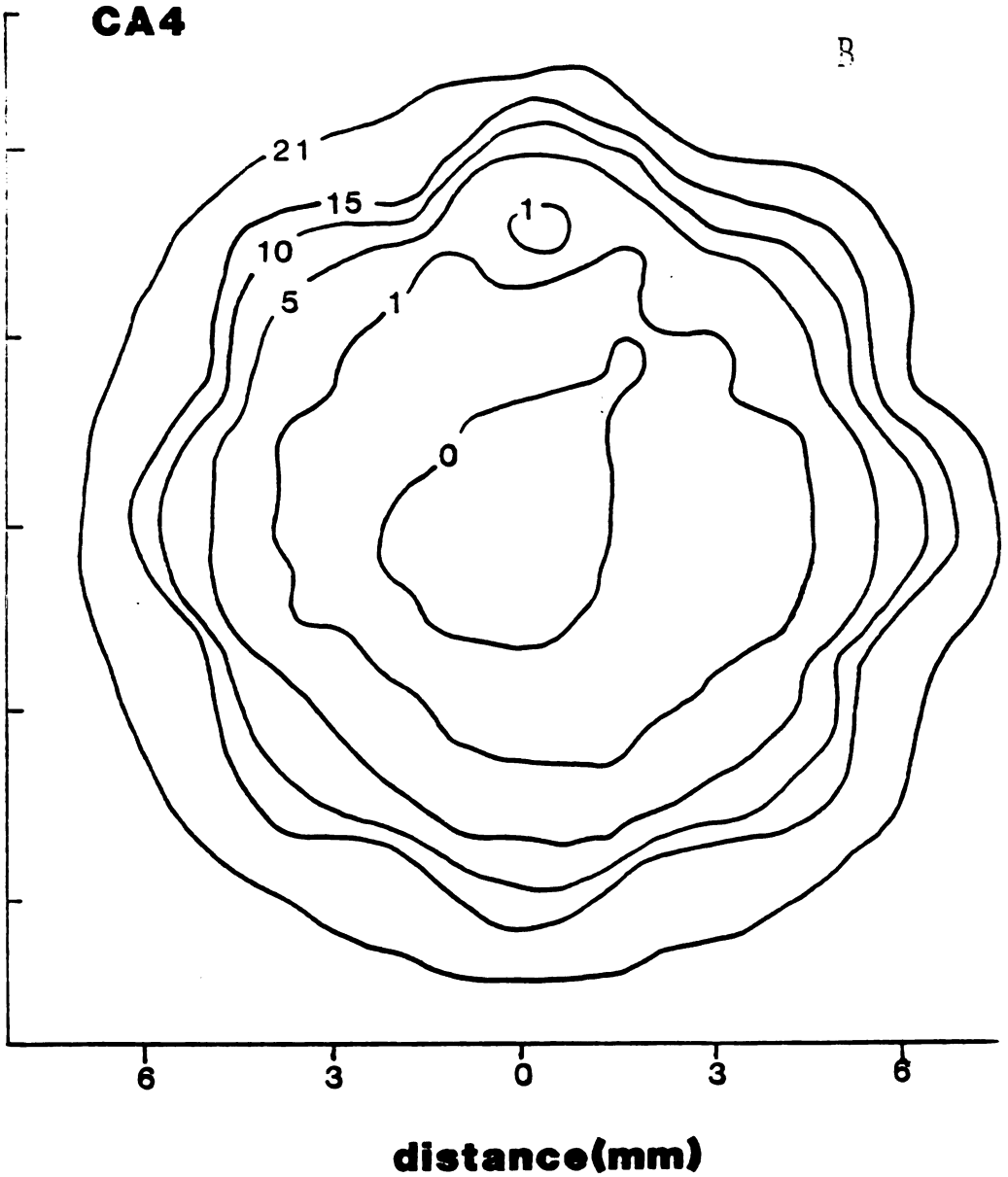

Figure 4 continued

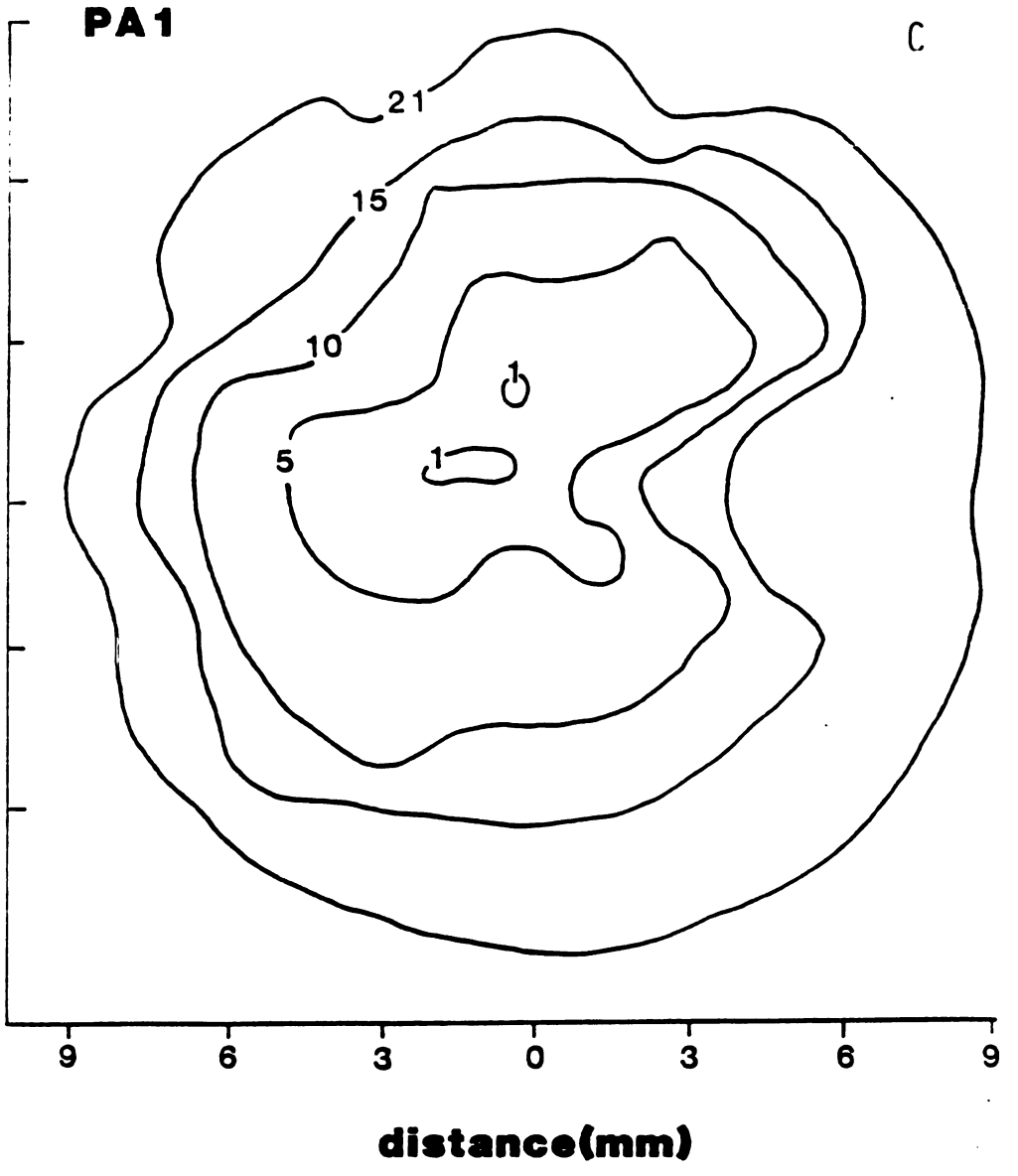

Figure 4 continued

Figure 5. Oxygen diffusion in aggregates. Shown are the recorder output of O_2 concentration at 0.4, 0.8, 1.2 mm below the surface of the aggregate. The point of change in the atmosphere surrounding the aggregate from water saturated air to 100% O_2 is indicated by the dotted line.

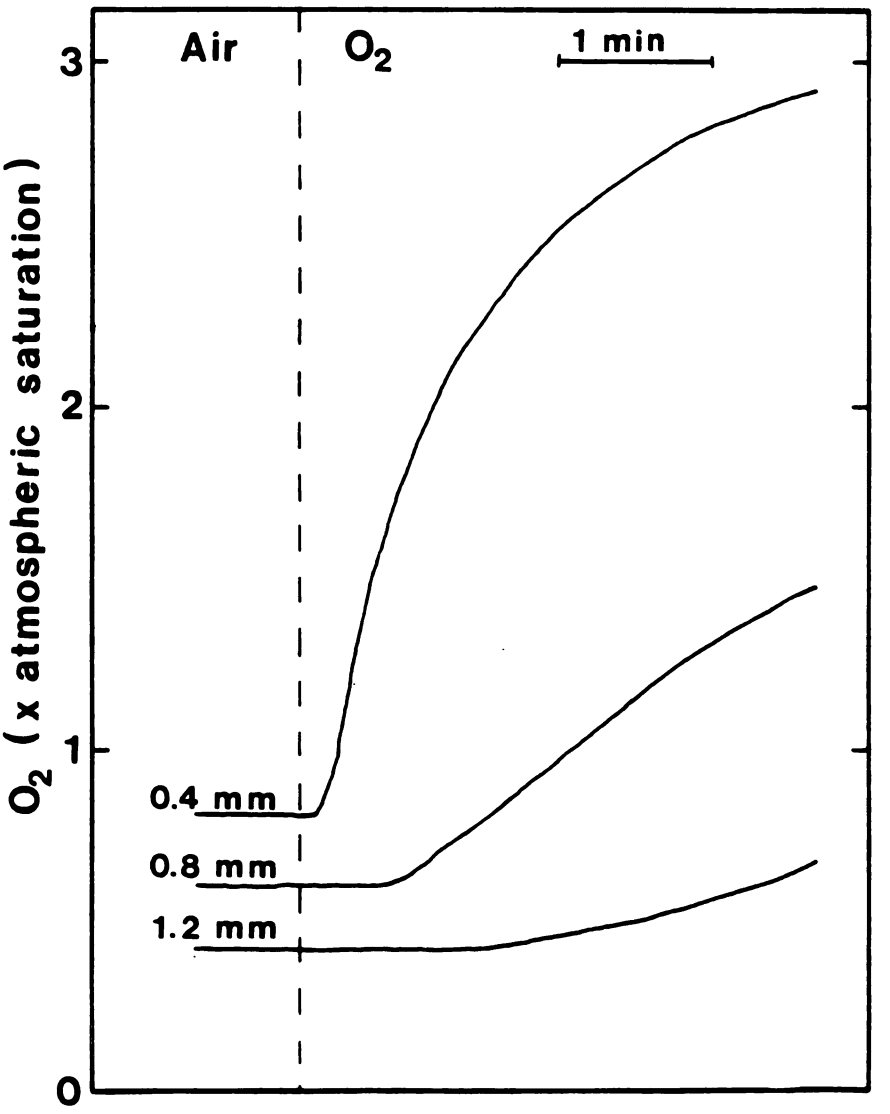

Figure 5

Figure 6. Error function graph, constructed according to Duursma and Hoede (1967), used to estimate the intra-aggregate O_2 diffusion coefficient from the relationship: where $C_{(x,t)}$ is the oxygen concentration at depth x(cm) and time t(200 sec) after rapidly changing the oxygen concentration to 100% O_2 (0) or 100% N_2 (●) at the aggregate surface. The radiating lines are theoretical $C_{(x,t)}/C_o$ values for different values of the diffusion coefficient (D_a) and are used to estimate the measured value of D_a by interpolation.

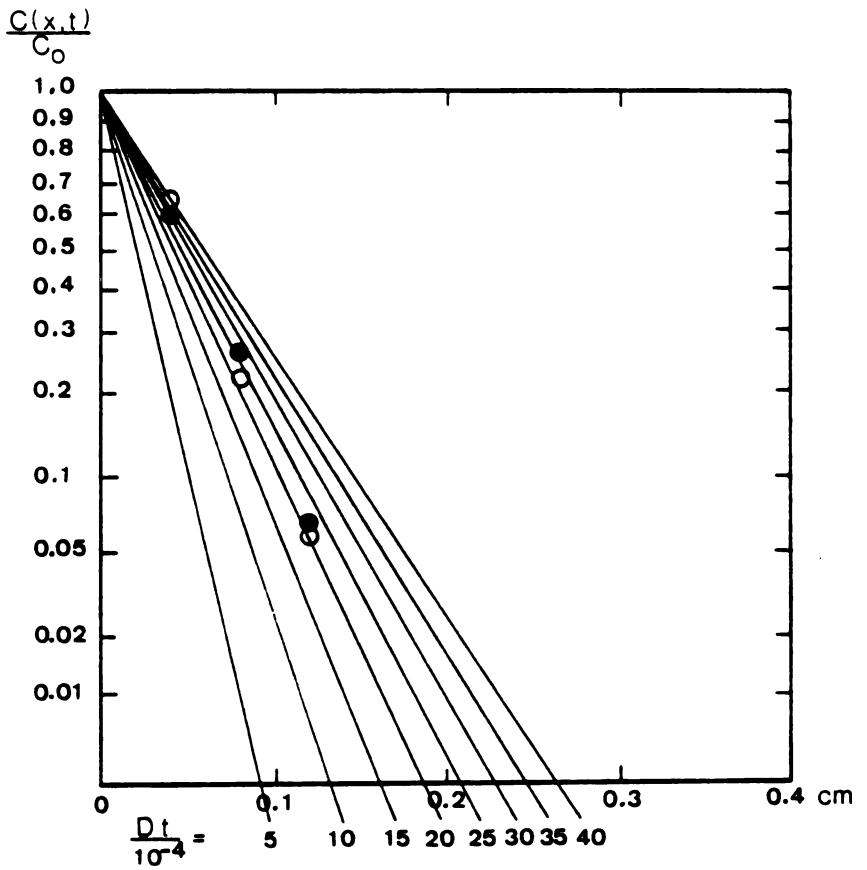
C(x,t)/C0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.05
0.02
0.01
0
0.1
0.2
0.3
0.4 cm
Dt/10^-4 =
5
10
15
20
25
30
35
40

Figure 6

always had a demonstrable anaerobic zone, however, some aggregates with anaerobic centers showed no denitrification. Aggregates obtained from a cultivated field (CA) had higher respiration than did aggregates obtained from native prairie (PA). The oxygen consumption rates calculated from the measured oxygen fluxes were generally less than those determined by CO_2 production, but agreed well with values reported for other soils at similar carbon contents (Greenwood, 1975). These values were used to calculate the anaerobic radius predicted by expressions derived by Smith (1980). The predicted anaerobic radius was similar to the average radius for the anaerobic zone measured by the oxygen microelectrode, and where different, the model tended to underestimate the radius. The magnitude of the denitrification rate did not correspond to aggregate size or to the anaerobic radius, indicating that factors other than anaerobic volume alone were determining the observed denitrification rates.

Figure 7. Values of $C_{(x,t)}$ obtained by numerically integrating Fick's second law using different values of D_a. Solid line represents the oxygen concentrations experimentally measured as described in Figure 5. The symbols correspond to different values of Da ($\times 10^{-6} cm^2 \cdot s^{-1}$) used in the numerical integration: were 11, 7, 5.9, and 3.

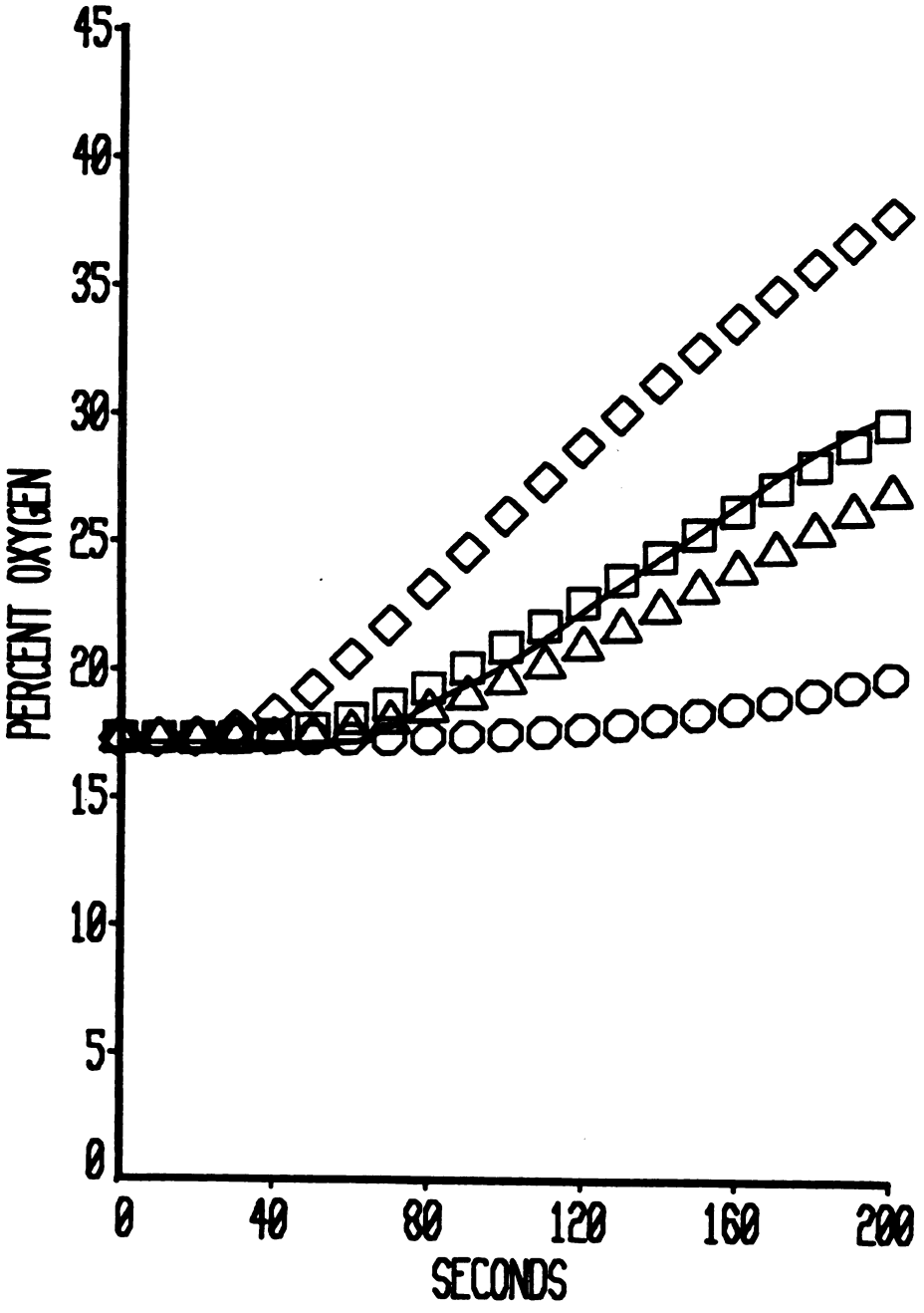
45
40
35
30
25
20
15
10
5
0
PERCENT OXYGEN
0
40
80
120
160
200
SECONDS

Figure 7

Table 1. Comparison of denitrification rate, respiration rate, and anaerobiosis in aggregates from cultivated and prairie soils.

Aggregate	Denitrification rate -(ng-N·g^{-1}·day^{-1})-	Respiration rate -(ml·ml^{-1}·sec)-	Oxygen flux -(ml·ml^{-1}·sec)-	Radius -(mm)-	Anaerobic Radius	
					Measured* -(mm)-	Calculated -(mm)-
CA4#	6.3	6.2	7.6	7.0	3.3	2.1
CA1	13.0	6.1	3.4	8.0	1.3	-
CA2	9.2	4.3	3.9	12.0	5.5	5.7
CA3	2.3	n.d.	4.3	13.0	5.4	7.2
PA1	0.03	2.9	1.6	9.0	0.0	-
PA2	1.7	6.1	1.2	18.0	14.0	5.6

* mean radius measured on 4 to 10 profiles on each aggregate

obtained from a cultivated silt loam (CA) or from adjacent uncultivated native prairie (PA)

- calculation indicates that no anaerobic radius should occur

n.d. - not determined

DISCUSSION

The oxygen sensing tip of the microelectrodes used in this study is much smaller than in any oxygen electrodes previously used in soil. The microelectrodes were made more sturdy for use in soil by increasing the wall thickness of the of the outer glass capillary. This could be achieved without effecting the inner diameter of the membrane or the size of the inner cathode. Although the electrode tips are flexible, they are still quite fragile because of their small size. In this initial study we chose to work with a soil containing little sand, since individual sand particles are much larger than the microelectrode and may damage the sensing tip. We worked only with wet aggregates to facilitate insertion of microelectrodes. Successful oxygen measurements were made in aggregates at moisture contents as low as 28%. In future work it will be important to determine whether anaerobic sites exist in drier aggregates, since we know that denitrification, can occur in soils at lower moisture contents than were used in this study. The measurement of oxygen under drier soil conditions will probably be limited to one profile in each aggregate, as the air channel created when the electrode is withdrawn will not refill with water as it did in our wet aggregates.

Greenwood & Goodman (1967) have provided the only other direct measurements of oxygen profiles in aggregates; they used 1 cm diameter, KCl saturated spheres. They concluded that their

electrode tip caused compaction when inserted into the aggregates causing steeper oxygen profiles. The tip of their electrode was 0.5 mm, 100 times larger area than the electrode used in this study. They measured complete anaerobiosis within 2 mm of the surface of their aggregate constructs. Their result is consistent with the observation that oxygen profiles in rolled or disturbed aggregates are steeper.

The profiles shown in Fig. 3 illustrate the necessity of measuring oxygen concentrations with high spatial resolution within soil aggregates. Steep oxygen gradients occurred in most aggregates over very small distances from the aggregate surface. Greenwood predicted that the smallest aggregate containing an anaerobic center would have a radius of 9 mm, assuming a D_a of 1 x $10^{-5} cm^2 \cdot s^{-1}$ and a respiration rate of 4.31 x $10^{-7} ml \cdot ml^{-1} \cdot s^{-1}$ (Greenwood, 1975). We have measured anaerobic zones in aggregates with radii as small as 4 mm. The respiration rates determined for the aggregates in our study, were similar to those assumed by Greenwood, however, we measured a lower average oxygen diffusion coefficient of 8.5 x $10^{-6} cm^2 \cdot s^{-1}$. Our mean diffusion coefficient agrees well with the range of values predicted for water saturated aggregates (Smith, 1980).

We used an integrated form of Fick's second law and the derivative form of Fick's first law describing one dimensional diffusion to obtain estimates of the intra-aggregate diffusion coefficient and the total aggregate oxygen flux, respectively. Although the aggregates were spherical, use of these simplified calculations was appropriate, since the radii of the aggregates

($\geq$ 8 mm) were large relative to the very small sensing tip of an electrode (1 to 3 μm) and the shallow depth of electrode insertion (0.35 to 1.2 mm). On this scale, oxygen would arrive at the inserted electrode tip, diffusing from an apparently planar surface boundary. Previous workers have successfully described steady state oxygen profiles in water saturated aggregates using one dimensional diffusion (Greenwood and Goodman, 1967), but they also included an oxygen consumption term in their formulation. Our estimates of D_a did not account for oxygen consumption in the aggregate since we assumed to be insignificant during the short duration (200 sec) of the experiment. This ommission could cause a slight underestimation of D_a. The results of numerically integrating Fick's second law over a range of D_a values to provide estimates of oxygen concentration with time at a fixed depth in an aggregate are shown in Fig. 7. Empirically comparing these results with oxygen concentration vs time of an actual experiment show that our experimental estimate of D_a appears to provide a good fit with the data, and thus give us confidence that our assumptions were reasonable.

The contour maps (Fig. 4) provide a two dimensional visualization of the oxygen profiles and anaerobic centers that occur in soil aggregates. Anaerobic zones were often assymmetric and did not always occur at the aggregate center. This suggests that sites of oxygen consumption within the aggregate are not uniformly distributed, presumably due to a non-uniform distribution of organic carbon within the aggregates. Because of

this anisotropy, a single oxygen profile or a two dimensional composite of oxygen profiles will not necessarily give a complete picture of aggregate anaerobiosis. As an example, the aggregate pictured in Fig. 2 B has no measurable zone of complete anaerobiosis in the two dimensional slice pictured, however, profiles obtained from at 90 degrees to the composite plane did contain an anaerobic zone, explaining the low but measurable denitrification rate obtained from this aggregate. A detailed description of anaerobiosis within a single aggregate will require many profiles obtained in all three dimensions.

Presence of an anaerobic profile was necessary before a denitrification rate was measured on any aggregate, however, some aggregates with anaerobic centers exhibited no denitrification. An anaerobic region of soil alone is not sufficient for denitrification. Soil denitrifiers also need a source of carbon as well as the presence of sufficient nitrate. Furthermore they may not be present at all anaerobic sites. Current mathematical models rely on the measurement of the soil anaerobic volume to subsequently predict the denitrification rate (Leffelaar, 1979; Smith, 1980). Measured denitrification rates in this study did not necessarily correlate with aggregate size or anaerobic radius, however, we have not yet made both measurements on a sufficient number of aggregates to evaluate a reliable correlation. It seems likely, however, that a spatial description of carbon and NO_3^- distribution will also be necessary before denitrification within one aggregate can be sufficiently predicted.

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APPENDIX A

THE EFFECT OF LONG TERM SOIL ACIDITY ON SOIL DENITRIFICATION RATES

Decreasing pH is generally observed to decrease denitrification rates, both of known denitrifying bacteria, and in soil. Observed pH optima generally occur between pH 7.0 and 8.0 (Bollag et al., 1970; Valera and Alexander, 1961; Nommik, 1956). Klemedtsson et al. (1978) observed that nutrient additions to an acid peat soil had no effect on denitrification rates, however increasing the pH from 3.5 to 6.5 greatly stimulated denitrification. Other workers have measured significant denitrification rates in acidic soils (Van Cleemput and Patrick, 1974; Gilliam and Gambrell, 1978; Muller et al., 1980). It is not clear whether the denitrification rate measured in very acidic soils is the result of small populations of denitrifiers in protected microsites of neutral pH, of general populations of denitrifiers functioning poorly in low pH environments, or of populations of denitrifiers with low pH optima (Firestone, 1982).

Measurement of biological denitrification in soil under acidic conditions is complicated by chemical instability of NO_2^- and the potential reactions of this compound with ammonia (Broadbent and Clark, 1965); amino acids and complex organic species (Soulides and Clark, 1958; Stevenson et al., 1970), and Fe^{2+} and Cu^{2+} ions in soil (Buresh and Moragham, 1976; Moragham and Buresh, 1977; Wullstein and Gilmour, 1966). These reactions are summarized by Knowles (1981). The gaseous products that have been reported from these reactions include NO, N_2O, N_2, and NO_2. In this study we report the effect of long term soil acidity on the pH option of denitrification activity in these soils eis reported.

MATERIALS AND METHODS

The site sampled for this study was a 15 m x 30 m area of Spinks sandy loam soil located on the Michigan State University farms, East Lansing, Michigan. This site was established in 1959, to assess the effect of various nitrogen carriers on soil acidification (Wolcott, 1965). Fertilizer treatments were continued until 1977 and resulted in subplots of pH as low as 3.7. In 1965, one-half of the study site was limed, restoring the soil pH to rear pretreatment levels.

The site was sampled during August 1982, as part of a larger study to evaluate spatial variablity of soil denitrification rates (Parkin et al., 1983). Soil cores (4.5 cm diameter x 25 cm long) were collected at 60 cm intervals along four transects, which ran between areas of low and high pH. Additional bulk soil was also collected and refrigerated at 4°C for later analysis.

Each core was coarsely sieved (0.5 cm) and used to prepare a soil slurry. Twenty-five grams of soil and 50 ml of distilled H_2O was added to a 250 ml Erlenmeyer flask. Each flask was attached to the previously described gas recirculation system and flushed with argon for 15 to 30 minutes to achieve anaerobiosis. Acetylene was added (20% v/v) and the slurry stirred while a denitrification rate was determined over a period of 1 to 2 hours. The denitrification rate was measured by following increasing concentrations of N_2O by gas chromatography. This assay is similar to that described by Smith and Tiedje (1979), and can be used as a measure of the activity of existing denitrifying enzymes in soil. Soil pH was determined in 2:1 slurries with 1N KCl, and NO_3^- concentrations were determined in 1N KCl extracts on a Technicon auto

analyzer.

A similar assay was performed using the bulk soil samples in which the pH of the soil had been adjusted. Soil pH was increased variously by adding saturated $CaCO_3^-$, 1 N NaOH or 25 mM MOPS buffer; and decreased using additions of 1% HCl or H_2SO_4 prior to the denitrification assay. Flasks were autoclaved and 1 mM NO_3^- added prior to rate determinations. A second set of controls was run using 3 gm of low and high pH soil and 3 ml H_2O in anaerobic culture tubes. Soils were autoclaved twice prior to gassing with argon and addition of acetylene. Replicates of non-sterile soils were also run. $NaNO_2^-$ was added to a final concentration of 1, 0.1, 0.01, 0.001 mM. Headspace gases were sampled at 8 and 24 hours.

RESULTS AND DISCUSSION

Denitrifying enzyme activity for the site ranged from 100 to 20,000 ng-N $g^{-1} \cdot day^{-1}$. Areas of low pH (< pH 5) averaged 1800 ng-N $\cdot gm^{-1} \cdot day^{-1}$, while areas of higher pH averaged 8500 ng-N $\cdot gm^{-1} \cdot day^{-1}$. The correlation of ln enzyme activity and pH was significant; $r = 0.661$ at $P < .001$ (Fig. 1). Other workers have observed similarly significant correlations (Muller et al., 1980; Bremner and Shaw, 1958; Dubey and Fox; 1974), while some workers have observed no correlation of denitrifying activity and pH (Khan and Moore, 1968; Burford and Bremner, 1975).

The establishment of long term low pH values at these sites may have selected for acid tolerant denitrifiers. Soils from areas of low pH (3.9) exhibited highest denitrification rates at a pH of 3.8, while soils from higher pH sites (6.8) exhibited highest rates at pH 7 (Fig. 2). This relationship was observed using several treatments to adjust the soil pH, suggesting that the observed optima was not caused by a specific chemical addition. Since the soil was a largely unaggregated sandy loam and was slurried, most organisms should not have been protected from the pH of the solution suggesting that microsites of high pH were not responsible for the observed rates.

Previous workers have observed chemical decomposition of NO_2^- in acid soils. Natural nitrate pools prior to imposed anaerobiosis ranged between 5 and 30 $\mu g\text{-}N \cdot g^{-1}$. Initially high rates of nitrate reduction in the slurry assay could cause transient accumulations of NO_2^- which, through chemical decomposition, might account for the N-gas observed in the low pH samples. The dominant products attributed to chemical

decomposition of NO_2^- reduction are NO and NO_2, with little N_2O found unless metals are involved (Moragham and Buresh, 1977). Sterile soils from the low pH subplot incubated in the presence of 1 mM to 1 μM NO_2^- exhibited no detectable N_2O over a 24 hour period. Non-sterile soils exhibited N_2O production rates of 2000 ng-N•g•day from NO_2^-at pH 3 indicating that biological reduction was the dominant fate in these soils. The low pH optimum for denitrification in acid soil suggests that acidopholic denitrifiers have been selected for by the 24 year period of decreasing pH.

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Figure 1. Linear correlation of denitrifying enzyme activity and soil pH from 204 samples of a sandy loam soil.

Figure 1

Figure 2. Influence of pH on denitrification enzyme activity in soil collected from areas of low and high pH.
□ = pH 3.9; 0 = pH 6.8.

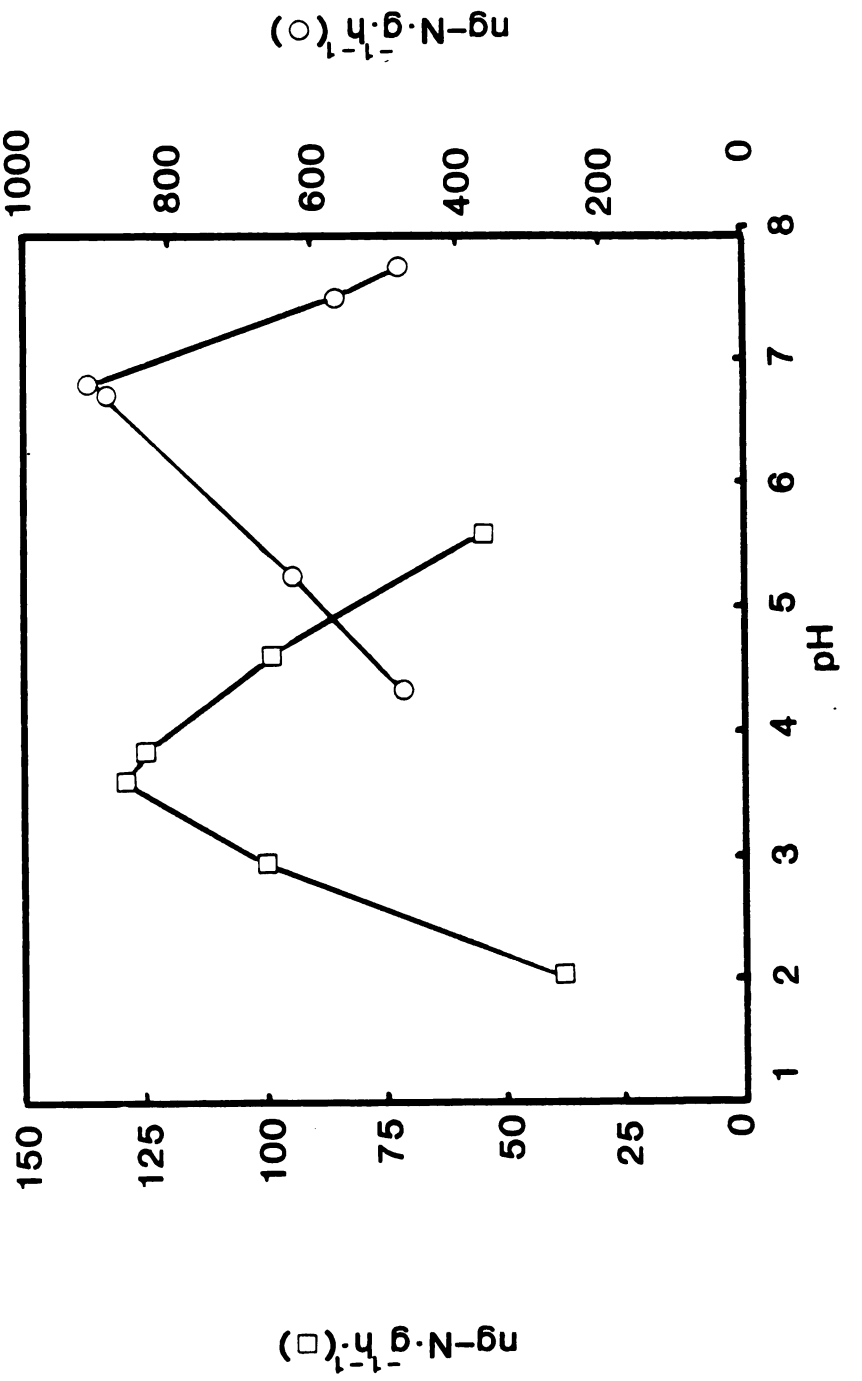
ng-N·g⁻¹·h⁻¹(○)
1000
800
600
400
200
0
150
125
100
75
50
25
0
1
2
3
4
5
6
7
8
pH
ng-N·g⁻¹·h⁻¹(□)

Figure 2

APPENDIX B

OXYGEN FLUX MEASURED WITH PLATINUM ELECTRODES AS RELATED TO SOIL DENITRIFICATION RATES

Lemon and Erickson (1952) described construction of bare platinum electrodes for measuring oxygen diffusion rate (ODR) in soil. The theory of operation of these electrodes is simlar to that described in Chapter III. The platinum surface is rendered negative at 0.5-0.8 volts relative to an external Ag/AgCl reference electrode. The resultant potential causes O_2 to be electrochemically reduced on the platinum surface. The surface area of platinum is relatively large (0.06 cm diameter x 0.4 cm long), and consumes oxygen from the surrounding media when a potential is applied. The electric current recorded by these electrodes is proportional to the rate of O_2 arrival at the electrode surface. This oxygen flux is related to the current by the following expression (Stolzy et al., 1981):

$$i \times 10^{-6} = n\,FAJ$$

where i is the current in microamperes, n is the number of electrons required to reduce one molecule of O_2, F is the Faraday constant, A is the electrode surface area, and J is the flux of O_2 ($umoles\text{-}O_2 \cdot cm^{-2} \cdot sec^{-1}$). The oxygen flux J, will vary with soil oxygen concentration and moisture content and can be used as an indication of soil aeration status (Phene et al., 1976). Fluhler et al. (1976) has suggested that ODR measurements provide a statistical approach for estimating the effect of anaerobic microsites on the soil denitrification rate.

Limitations of the method have been discussed in detail by McIntyre (1970). The current observed is affected by the voltage applied (Va). It is necessary to calculate an effective applied voltage

(Ve) by taking into account the resistance (R) between the platinum electrode and the Ag/AgCl reference:

$$Ve = Va - R$$

It is also necessary to physically separate the reference electrode from the measuring electrode by a distance of 5-10 cm to obtain reliable readings. The electrode surface can be poisoned by soil constituents and must be frequently cleaned. Finally, the method is appropriate only in relatively wet soils, since a water film must cover the electrode surface and be continuous with the reference electrode.

Electrodes were constructed according to Van Dorn and Erickson (1966) to evaluate relationships between ODR values and observed denitrification rates. Soil cores for denitrification measurements in Chapter I were modified with side ports such that 10 electrodes could be inserted through gas tight rubber septa. The Ag/AgCl reference was placed in contact with the soil surface through a rubber stopper at the top of the core. The soil core could be attached to the gas recirculation system and ODR measurements and denitrification rates simultaneously determined. Experiments were performed on a coarsely sieved clay loam soil packed in the cores to a bulk density of 1.5 Mg/m^3 and wetted to moisture contents ranging from 22 to 30%.

ODR values were determined using a Jensen Instruments Model 13 ODR rate meter. Resistance between each electrode and the reference was determined using a conductivity bridge (Bornstein and McGuirk, 1978). Relationships were constructed between resultant current and applied cathode voltage (Fig. 1) and were used to calculate the current at V_E = 0.5 volts.

Figure 2 shows ODR values and denitrification rates determined at varying pore space oxygen concentrations. The reported ODR value is the

mean flux determined from 10 replicate electrodes. The ODR rate decreased rapidly as the %AR begun to increase at low pore space oxygen concentrations. In an earlier study, Brandt et al. (1964) also observed denitrification rates in a clay loam soil at ODR values of less than 0.2 $g\text{-}O_2 \cdot cm^{-2} \cdot min^{-1}$. Differences in ODR values measured in soils of varying moisture contents were observable at pore space oxygen concentrations of 18% (Table 1) suggesting that ODR values, in addition to soil moisture content might be used to predict soil denitrification rates. Differences were not discernable at lower O_2 concentrations. Measured differences in the mean ODR values at the various moisture contents were small (0.2 to 0.5 $\mu moles\text{-}O_2 \cdot cm^{-2} \cdot min^{-1}$, with sufficient variability among electrode measurements (CV = 10 to 30%) to obscure differences. This variability may result from the large physical size of the electrode, which is in contact with many potentially different aeration sites. ODR values provide an additional indirect measure relating soil aeration to denitrification rates. The measured flux cannot, however, be used to quantitatively determine oxygen diffusion coefficients, which as discussed in Chapter II and III is the important parameter necessary to describe soil anaerobiosis and its effect on denitrification.

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Table 1. The effect of increasing soil moisture on oxygen flux and denitrification rates in a clay loam soil.

moisture content	oxygen flux	% anaerobic rate
(%)	(μmoles-O2$\cdot$cm$^{-2}\cdot$min^{-1})	
22	0.47 $\pm$ .06	0.7
24	0.35 $\pm$ .06	0.9
26	0.31 $\pm$ .04	2.1
30	0.28 $\pm$ .03	1.9

Figure 1. Relationship between electrode current and applied effective voltage used to calculate current at effective voltage of 0.5. Examples from 3 electrodes in a clay loam soil at 26 percent moisture.

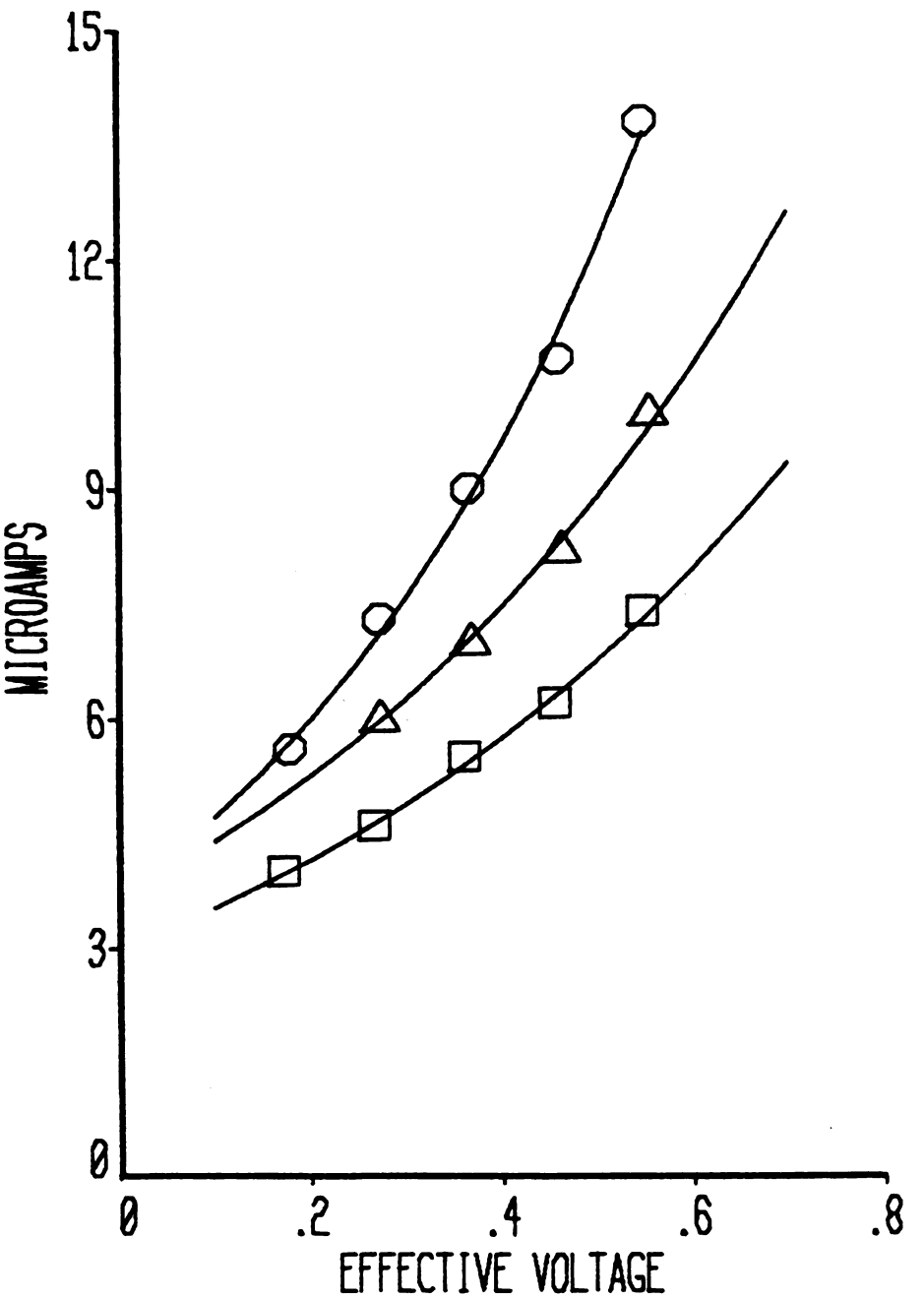

Figure 1

Figure 2. Relationship between percent anaerobic rate, oxygen flux, and pore space oxygen concentration in a clay loam soil at 26 percent moisture.

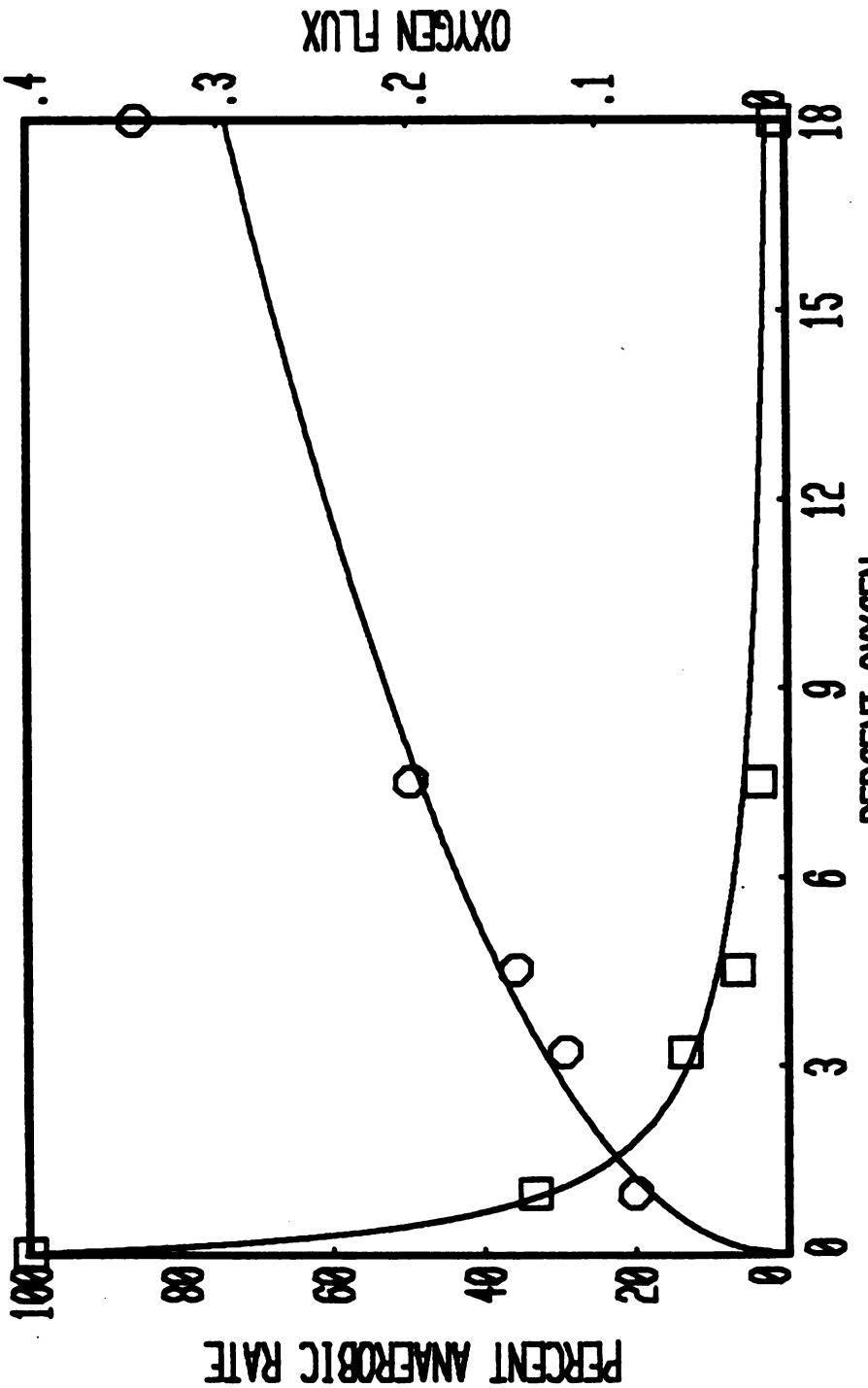
OXYGEN FLUX
.4
.3
.2
.1
0
18
15
12
9
6
3
0
PERCENT OXYGEN
100
80
60
40
20
0
PERCENT ANAEROBIC RATE

Figure 2