Plant and Soil 76, 197–212 (1984). Ms. BPSF 4.1

Anaerobic processes in soil

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Key words Aggregate oxygen profile Anaerobic radii Anaerobiosis Anoxia Aromatic ring cleavage Clostridium Dechlorination Denitrification Denitrification pathway hypothesis Fermentation Fe^{3+} Mn^{4+} reduction Methanogenic microorganisms ^{13}N ^{15}N N–N bond N_2O NO_3^- respiration/dissimilatory reduction O_2 consumption O_2 diffusion coefficient O_2 micro electrode Pesticides Reduction NO_3^- NO_2^- SO_4^{2-} CO_2 H^+ Soils flooded poorly and well drained Sulphate Xenobiotics

Summary Anaerobic conditions in soil affect plant productivity as well as organic matter and nutrient dynamics. Anaerobic processes often dominate biological and chemical features of flooded and poorly drained soils but in well-drained soils, anoxia is restricted to small zones and to limited periods. The anaerobic processes listed according to their approximate sequence of occurrence as the redox decreases are: Fe^{3+} and Mn^{4+} reduction, denitrification, fermentation, nitrate respiration, dissimilatory nitrate reduction to ammonium, sulfate reduction, carbon dioxide reduction, acetate splitting, and proton reduction. Two of the anaerobic processes, denitrification and fermentation of pollutant chemicals have been studied and recent results are summarized here. We describe the measurement of denitrification using a recirculating atmosphere of acetylene, provide quantitative information on the effect of moisture and oxygen on denitrification, and report on the oxygen concentration within soil aggregates measured by oxygen microelectrodes. The current hypotheses for the pathway leading from nitrate to the N–N bond and N_2O are also presented. Recent work in our laboratory has shown a new reaction for the metabolism of some chlorinated organic chemicals. In this reductive dehalogenation, the ring Cl is replaced by a proton. An unusual organism which carried out this reaction has been enriched and isolated on 3-chlorobenzoate. These anaerobic reactions may be of further use in pollutant removal. With these new techniques and knowledge available, it is now possible to gain a better understanding of the biochemistry, physiology, ecology and diversity of the anaerobic organisms and their processes in soil.

Introduction

Most cultivated soils can be considered as being dominated by aerobic conditions, but there are situations when anaerobiosis prevails which can be harmful to plant productivity. Detrimental effects of anaerobiosis include roots stressed by anoxia; loss of N by denitrification; production of organic acids, H_2S, and perhaps other plant toxicants; and favored conditions for the development of root pathogens. But, not all effects of anaerobic conditions are detrimental. Beneficial aspects of anaerobiosis are: N_2 fixation by free-living heterotrophs is usually more significant under anaerobic conditions; mineralization of organic matter is retarded; certain pesticides, *e.g.* DDT[19]

and lindane, are more rapidly degraded; and anaerobiosis can be effective in control of certain plant pests.

Anaerobiosis occurs in soil when the oxygen consumption rate exceeds the supply rate. The rate of oxygen consumption depends primarily on the amount of available carbon for respiration with its rate of use being regulated by water availability and temperature. The oxygen supply rate depends on the moisture content and the physical characteristics of the soil, especially porosity which is influenced by structure and texture. A change in either the rate of oxygen consumption or supply can bring about anaerobiosis, *e.g.* a large application of manure or by compaction of the soil. Most soil anaerobiosis is caused by high soil moisture resulting from a high watertable or heavy rains. The dramatic effect of water on the aeration status is due to several factors: the much lower oxygen diffusion coefficient of water filled pores than of air filled pores, a much smaller reservoir of oxygen in soils with a high proportion of water filled pores, and a stimulation of respiration caused by solubilization and movement of nutrients and resurgence of metabolism after dessication.

Soils exhibit a continuum from those that are flooded and continuously anaerobic to the sandy, excessively drained soils for which it is hard to imagine any anaerobic microsites. For simplicity, however, it is reasonable to consider three major classes: the flooded soil (*e.g.* paddy rice culture), the poorly drained soils, and the well-drained soils. The flooded soils will have a few to several millimeters of aerobic soil overlaying deeper soil that remains anaerobic for the duration of flooding[6]. In this situation it is expected that all anaerobic microbial processes can occur. The poorly drained soils have significant periods of anoxia caused by a high watertable in the spring and following heavy rainfalls when temporary anaerobic sites are created. The well drained soils may have limited areas and periods of anaerobiosis; most likely when there are large, water saturated aggregates but it is unlikely that all the anaerobic processes occur, at least to any significant extent.

Anaerobic microorganisms and processes

The anaerobic microbial processes are summarily listed in Table 1 according to their approximate sequence of occurrence as the redox of soil decreases. The first four processes are those which might be expected in any soil with temporary anaerobic microsites, *e.g.* well-drained soils. The organisms that carry out these processes are all facultative anaerobes and thus easily make the shift from aerobic to

Table 1. Anaerobic microbial processes and their reaction products

Process	Reaction[a]
Fe^{3+}, Mn^{4+} reduction	$OM^{b} + Fe^{3+}, Mn^{4+} \longrightarrow Fe^{2+}, Mn^{2+}$
Denitrification	$OM + NO_3^- \longrightarrow N_2O, N_2$
Fermentation	OM $\longrightarrow$ organic acids, principally acetate and butyrate
Nitrate respiration	$OM + NO_3^- \longrightarrow NO_2^-$
Dissimilatory NO_3^- reduction to NH_4^+	$OM + NO_3^- \longrightarrow NH_4^+$
Sulfate reduction	OM or $H_2 + SO_4^{2-} \longrightarrow S^{2-}$
Carbon dioxide reduction	$H_2 + CO_2 \longrightarrow CH_4$, acetate
Acetate splitting	Acetate $\longrightarrow CO_2 + CH_4$
Proton reduction	Fatty acids and alcohols + $H^+ \longrightarrow H_2$ + acetate + CO_2

[a] The major reduction products are shown. Oxidized products are also produced; this is usually CO_2 if the electron donor is an organic compound.
[b] OM = Organic matter.

anaerobic growth. The first process, Fe^{3+} and Mn^{4+} reduction, is the only one for which no specific enzyme is responsible; these reductions are thought to result indirectly from other microbial products. The dissimilatory reduction of NO_3^- to NH_4^+ is carried out by both facultative and obligate anaerobes but this process is not prevalent in most soils probably because the carbon to electron acceptor ratio of soils is not sufficiently high to select for this reduction[35]. The last four processes are carried out by obligate anaerobes that are not expected to be significant in soils except under flooded or intensively anaerobic conditions. The last three processes in Table 1 occur when organic matter is converted to methane by lithotrophic bacteria that reduce CO_2 with H_2 to form methane, and by bacteria that split acetate into CH_4 and CO_2[2,28]. Proton reduction is carried out by acetogens that anaerobically oxidize butyrate and propionate to acetate[28]. These are the only known organisms that can utilize these organic acids in the absence of external electron acceptors. If methane is produced, usually these acetogens are also active: if not, the increased acidity would inhibit methanogenesis.

Organisms capable of anaerobic growth in soil include the facultative and obligate anaerobes. According to Skinner[19] the former are quite numerous representing up to 10% of the total soil population (*i.e.* 10^7 organisms per g soil) detected by plate count. It is the facultative anaerobes that are probably responsible for fermentation in the soils with the more temporary anaerobic conditions. The Clostridia are the most numerous obligate anaerobes and exist in densities of 10^3 to 10^5 organisms per g soil. Clostridial strains in soil can ferment carbohydrates (saccharolytic), proteins (proteolytic), hydrolyzed

cellulose, and fix N_2. It is thought that in dry soil Clostridia mostly exist as spores rather than as vegetative cells so they are generally inactive and slow to respond when conditions change. In poorly drained and flooded soils, they would be expected to play a more important role than in the more aerobic soils.

Soil scientists who do research with air dried, stored soils should be aware that this treatment favors spore forming organisms like Clostridia. When water is added to these dry soils, spore formers would be expected to have a proportionally greater impact on the soil metabolism than they would under field conditions.

Sulfate-reducing bacteria and methanogens are the other obligate anaerobes known to exist in soil, but soil is not considered a favored environment for their growth. Whether other obligate anaerobes exist in soil has not been investigated. Recent advances in anaerobic technique plus new interest in these little studied organisms has revealed a large number of new genera of anerobic heterotrophs, sulfate-reducing bacteria, and methanogens[2,9,28]. Whether any of these newly discovered organisms also exist in soil is not known, but there is no evidence that other obligate anaerobes are of practical importance in soil.

Microorganisms are considered anaerobic when they have a mechanism to generate energy (grow) other than by coupling electron transport to oxygen reduction. However, some anaerobes are also inhibited by oxygen; these are the obligate anaerobes. The reason for this inhibition is thought to be the production of toxic oxygen forms such as singlet oxygen (anti-parallel spin of the outer pair of electrons) and the reactive forms resulting from one electron reductions of oxygen illustrated by the reductive sequence shown in Fig. 1. These forms can occur during the respiratory reduction of oxygen and also from oxygen reaction with flavins, thiols, and other cell constituents. Aerobic organisms have one or more of the enzymes shown in Fig. 1 to remove these toxic intermediates while anaerobic organisms usually do not. However, for these toxic intermediates to be produced, there must be a flow of electrons. In soil where metabolism is severely restricted by the lack of available C, there may not be sufficient electron flow to generate these toxic intermediates. This may help explain why very oxygen sensitive organisms, such as methanogens, can survive in "aerobic" soil. Recently Kiener and Leisinger[10] showed that several methanogens were less harmed by oxygen when in the starved condition[10].

The presence of obligate anaerobes in a soil cannot be used to indicate the prevalence of anaerobic conditions since all three groups – Clostridia, sulfate-reducing bacteria, and methanogens – can survive

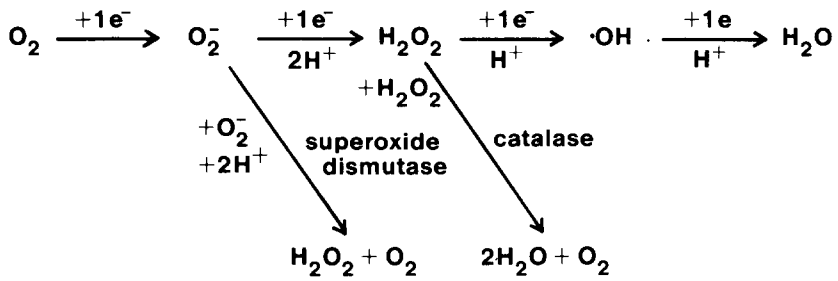

Fig. 1. The toxic intermediates formed from single electron reductions of O_2. The enzymes which protect aerobic organisms from these toxicants are also shown. Anaerobes often lack these protective enzymes.

well in the absence of anaerobic growth conditions. How long they can survive and how often they must experience anoxia to maintain their population is not known. It does seem, however, that the obligate anaerobes in soil are present in low numbers and show limited diversity relative to the known organisms capable of anaerobic growth, which supports the concept that most non-flooded soils are generally aerobic. Hence, the anaerobic processes that most likely occur in cultivated soils are those carried out by facultative anaerobes, the first four processes in Table 1. Of these, Fe^{3+}, Mn^{4+} reduction, denitrification and NO_3 respiration require the aerobic generation of the oxidized ions first which suggests that these processes should be more significant wherever there are more aerobic-anaerobic interfaces. Fig. 2 illustrates this interface. The anaerobic front in the aggregate is thought to expand and contract in response to the supply and respiratory consumption of oxygen. This concept suggests that denitrification, for example, should be more extensive in habitats where the area of the interface is greatest and where the movement of this zone is periodic.

Of the anaerobic processes in soil, denitrification and fermentation are of most practical importance and are further illustrated here with a summary of the current work from my laboratory. For complete information the reader should refer to our detailed papers by Parkin *et al.*[11,12,13], Sextone *et al.*[17,18] and Parkin and Tiedje[14].

Methods to study denitrification in soil

Denitrification has been a very difficult process to study because of the inability to measure the major product (N_2) in the atmosphere. We have used ^{13}N, ^{15}N, and the acetylene inhibition of N_2O reduction[3,24,27] to study denitrification since each method has unique advantages. ^{13}N, the longest lived radioactive isotope of nitrogen (half-life 9.96 min), is the only method where one can directly measure

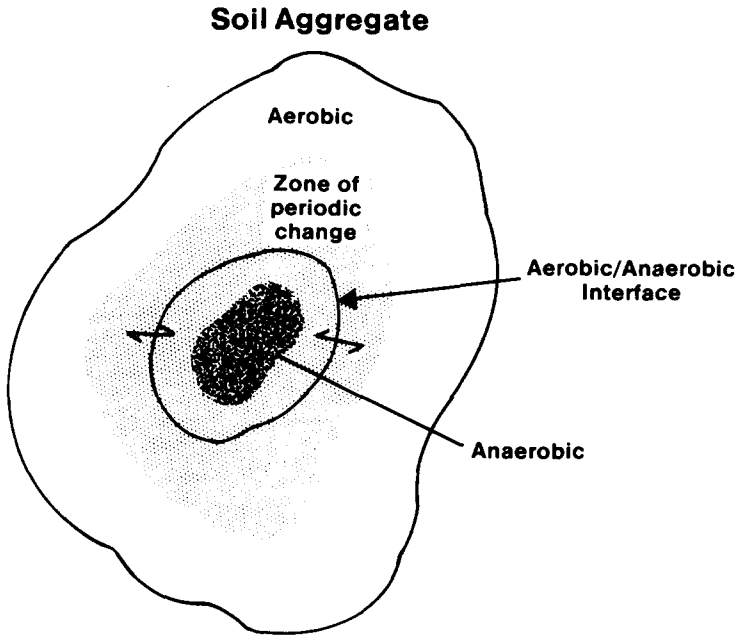

Fig. 2. Diagram of oxygen status of soil aggregate which illustrates how the anaerobic zone expands and contracts periodically in response to moisture changes. It is suggested that this dynamic movement is important for processes that require both aerobic and anaerobic conditions, *e.g.* denitrification.

the denitrification product in the natural atmosphere. Furthermore, ^{13}N allows very sensitive measurements (approximately 10^9 orders of magnitude more sensitive than ^{15}N for short-term measurements), and, because of this sensitivity, one can make labelled N additions that are well below the Km of the process thereby not affecting the natural rate. Use of ^{15}N is best suited for N balance experiments in the field where many samples are required and one wants to integrate information over time. The acetylene inhibition method is sensitive, can be used in the field, and, very important, relies on the natural NO_3 substrate concentration and distribution, something not possible by any label method. We believe that the acetylene method offers the most promise as a routine method to study denitrification in laboratory and field because of the number of samples that can be analyzed and the fewer assumptions required in interpreting the results. Acetylene is known to have other effects but the only one of significance to short-term studies of denitrification is the inhibition of nitrification[8,26] *if* the NO_3 concentration is very low.

We have developed a system using intact soil cores to measure natural rates of denitrification in which acetylene is distributed by gas flow. This method is more thoroughly described and evaluated

in Parkin *et al.*[11]. Briefly, acetylene plus oxygen at the concentration measured in the soil pores[14] is recycled through soil by means of a membrane pump. The rate of N_2O production is measured by electron capture gas chromatography, and, when this production is linear, it should reflect the rate of denitrification. The basic advantage of this acetylene method is that the forced air flow overcome the slow diffusion of acetylene into and N_2O out of the soil. Denitrification rate measurements can be made within 1 to 2 hours and thus the rate should reflect the soil conditions in the field before the sampled core has been significantly influenced by its removal from the environment. We have automated this method so that eight cores can be run simultaneously with up to 60 cores processed per day. Denitrification losses measured by this method were compared to ^{15}N losses determined by difference for cylinder microplots at two field sites. The losses determined by the acetylene method were 1.5 to 3 times less than the ^{15}N loss estimates although the 95% confidence intervals overlapped. This difference between the methods could be due to the higher moisture content of the soil in the ^{15}N microplots, to the inaccuracies and assumptions associated with measurement of total denitrification by ^{15}N, or to a greater proportion of aerobic microsites that might be caused by the forced gas flow. Our evaluations to date, however, have not shown a significant inhibitory effect of the forced air flow on denitrification[11]. Our experience with the ^{15}N difference method is that it is subject to more inaccuracies than the acetylene core method. The problems result from the difficulty of obtaining and maintaining a uniform label distribution; obtaining accurate, time-dependent measurements of ^{15}N ratios of the various N pools: and the required assumptions on the various fates and recycling of N.

The effect of oxygen and moisture on denitrification

We have used the acetylene recirculation method to measure the effect of oxygen concentration on denitrification (Fig. 3). The data on denitrification rate (circles) are expressed as a percentage of the anaerobic rate (using argon as recirculation gas) of the measured core, to normalize to a standard condition and to reduce the variability among cores[14]. The solid line in the figure was generated from the model of Smith[20] which predicts anaerobic volume of aggregates as a function of aggregate size, respiration rate (Q), pore space oxygen concentration, and the oxygen diffusion coefficient (D). The figure also shows the values of Q and D used to obtain this best fit line. Although we have not yet measured these values, the similarity in shape of the denitrification rate and the anaerobic volume as a function

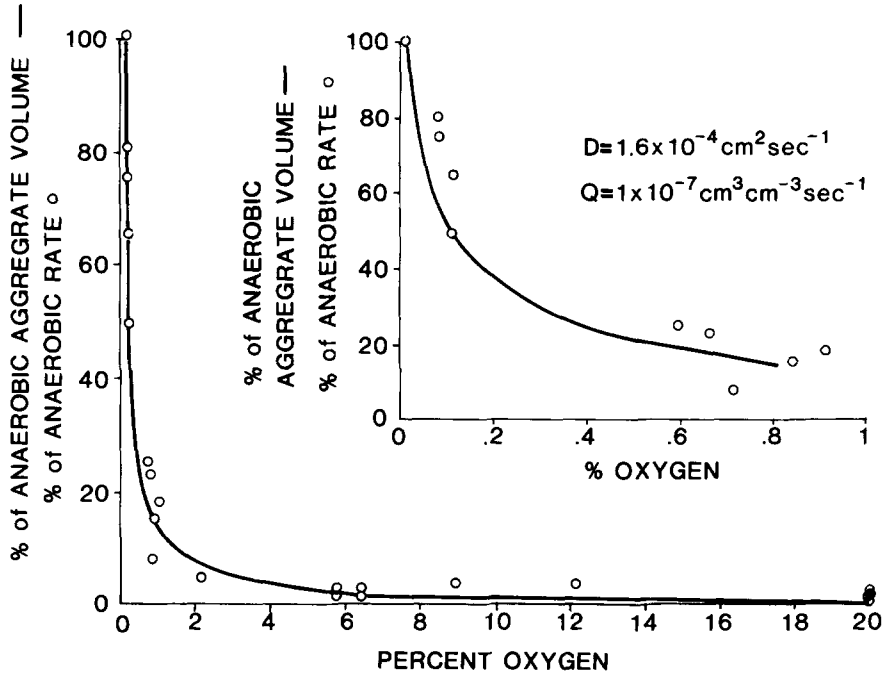

Fig. 3. Denitrification rates in a clay loam soil core at different pore oxygen concentrations (circles). Denitrification rates are expressed as a percentage of each core's anaerobic rate. The curve is percent of aggregate volume which was anaerobic as calculated by Smith's model of anaerobic volume. The inset shows an expanded view at low O_2 concentrations. Also shown are the diffusion coefficient of O_2 (D) and the O_2 consumption rate (Q) used to generate the curves. From Parkin and Tiedje[14].

of oxygen concentration suggests that the physical conditions imposed by the soil result in similar effects on the two measurements.

Fig. 3 also illustrates the strong inhibitory role of oxygen in restricting the activity of the denitrifying enzymes existing in soils. At 20% oxygen usually less than 5% of the potential activity was expressed. The denitrification rate increased as the oxygen concentration was decreased to below 2% where the rate increased dramatically. In nature, pore space oxygen rarely falls to this concentration in non-water saturated soils. Hence it seems unlikely that much of the potential denitrifying activity in soil is ever expressed.

We have used the acetylene core method to measure denitrification rates in the field and to study the effect of moisture on denitrification. These are detailed in the papers of Parkin *et al.*[11,12] and Sextone *et al.*[17] from this laboratory. As expected, denitrification often dramatically increased following rainfall or irrigation. In the two sites studied, 38 and 55% of the N loss occurred within 48 hours after moisture additions of greater than 1 cm[17]. This response to moisture shows

that it is important to accurately quantify denitrification N losses following rainfall and that sampling schedules must be determined by rainfall events if one hopes to achieve an accurate measure of N loss. We have also used geostatistical methods to determine whether there is a spatial component to the variability seen in denitrification rates. In the two sites analyzed so far we have found a spatial component sufficiently often to be encouraged that these techniques can be beneficially used in field studies of denitrification[13]. We have used punctual kriging to provide a contour map of denitrification rates for the study area and block kriging to provide improved estimates for denitrification losses over the block.

We have followed lines of British soil scientists – J. A. Currie, D. J. Greenwood, and K. A. Smith – interested in anaerobic microsites by extending their investigations to measurement of oxygen profiles within soil aggregates using a Clark-type, oxygen microelectrode. Revsbech and Ward[16] developed this electrode for work in hot springs and modified it to have thicker glass walls to be sturdy enough to insert into soil aggregates. Such electrodes have a 1 to 3 μm diameter membrane measuring tip and a response time of < 0.5 s, are stable for hours, and have a microscopic gold cathode so that the rate of oxygen consumption is insignificant. The sturdy electrodes used for soil aggregate had a shaft diameter of approximately 40 μm and were inserted at 0.1 mm increments with a micromanipulator. Details of the electrodes and results obtained with them are reported by Sexstone *et al.*[18]; some of the results are summarized here.

We studied aggregates from two Iowa silt loam soils, one in continuous cultivation for 50 years and the other from a nearby native prairie. The Iowa soil was chosen because of its low sand content since sand particles can damage or break the electrodes. The aggregates were moistened by placing them on water saturated filter paper for three days before study. Oxygen profiles within aggregates from the prairie and cultivated soil are illustrated in Figs. 4 and 5, respectively. The difference between these two profiles is greater than between populations of aggregates examined from the two sites, although the prairie aggregates had more root channels which caused more irregular profiles. These profiles illustrate that regular and irregular patterns can be measured by the electrode. As the electrode was withdrawn after insertion the oxygen readings during the withdrawal reproduced those measured on insertion which further confirm the accuracy of the method.

By inserting electrodes at different positions in a plane around the aggregate surface we were able to obtain contour maps of the

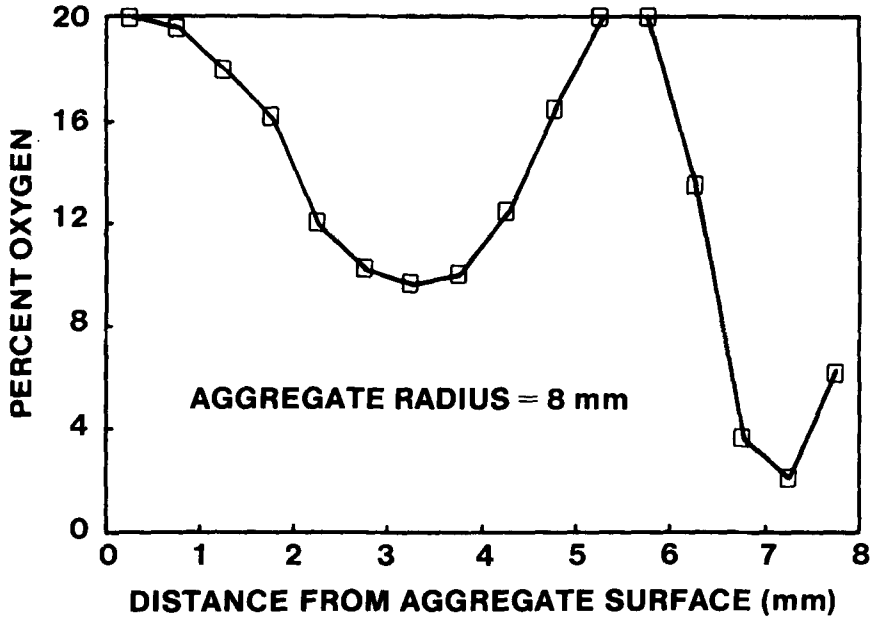

Fig. 4. Oxygen profiles within a silt loam aggregate from an Iowa native prairie soil. The discontinuity is due to an old root channel.

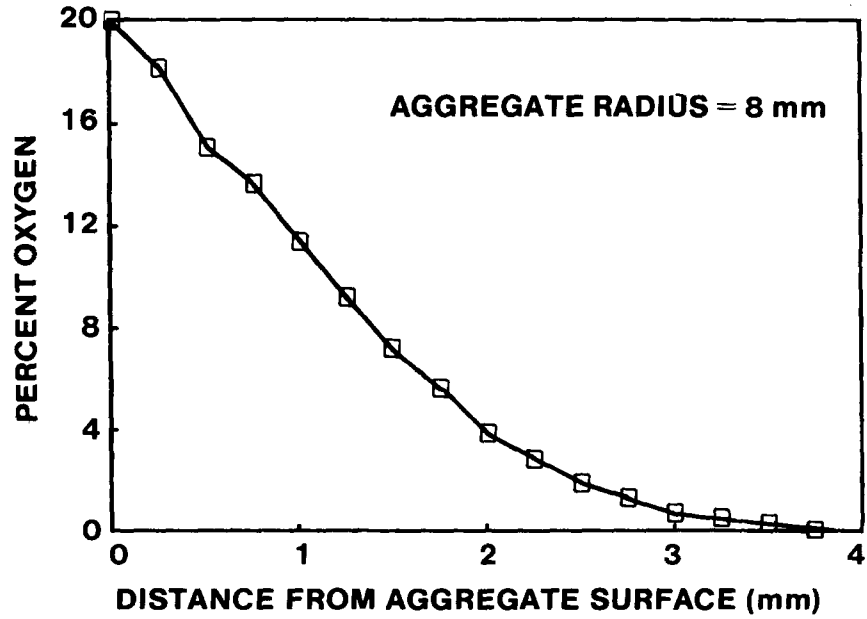

Fig. 5. Oxygen profile within a silt loam aggregate from an Iowa soil in continuous cultivation.

Table 2. Comparison of denitrification and anaerobic radii measured and calculated from cultivated and prairie soil aggregates. Taken from Sexstone *et al.*[18]

Aggregate	Denitrification rate ($ng - N \cdot g^{-1} \cdot day^{-1}$)	Aggregate radius (mm)	Anaerobic radius	
			Measured[a] (mm)	Calculated (mm)
CA4[c]	6.3	7.0	3.3	2.1
CA1	13.0	8.0	1.3	–[b]
CA2	9.2	12.0	5.5	5.7
CA3	2.3	13.0	5.4	7.2
PA1	0.03	9.0	0.0	–
PA2	1.7	18.0	14.0	5.6

[a] Mean radius measured on 4 to 10 profiles on each aggregate
[b] calculation indicates that no anaerobic radius should occur
[c] obtained from a cultivated silt loam (CA) or from adjacent uncultivated native prairie (PA)

oxygen profiles within aggregates[18]. These aggregates had radii of 6 to 9 mm; two of the three showed anaerobic regions near the center.

We also used the electrode to measure the intra-aggregate oxygen diffusion coefficient and, by gas chromatography, we have measured the aggregate respiration rate and denitrification rate. Smith[20] extended the models of radial diffusion to evaluate the extent of anaerobic zones within soil aggregates. Using his model and the measured oxygen diffusion coefficient, respiration rate, external oxygen concentration and aggregate radius we were able to calculate the predicted average anaerobic radii of the aggregates. As is shown in Table 2 there is good agreement between the measured and calculated anaerobic radii. The denitrification rates did not correlate well with the anaerobic volume, but too few aggregates have yet been measured to adequately assess this relationship. Denitrification rates were only measured in aggregates where a measureable anaerobic site was detected but not all aggregates with anaerobic sites denitrified.

Pathway to the N–N bond formed in denitrification

Despite the awareness of denitrification for a century it has never been resolved how the N–N bond is formed in denitrification. This is the key step in denitrification since it is the point of "no return" in which available N is lost to most of the biota. It is now well accepted that NO_2^- and N_2O are intermediates in denitrification, but the status of NO is less clear. NO is produced by all denitrifiers and thus must have some unique relationship to denitrification. Free NO does not seem to be an obligatory intermediate since in labeling experiments the N from NO_2^- reduction does not mix well with added NO[7]. However,

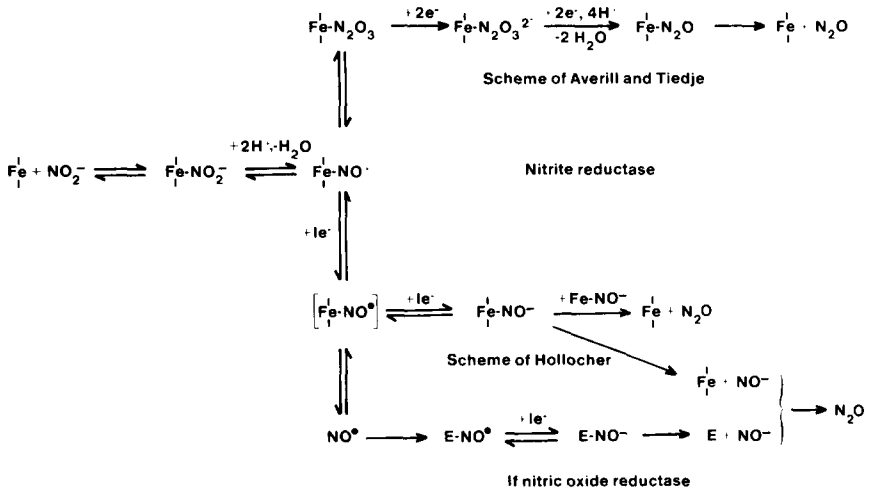

Fig. 6. Hypothesis of Averill and Tiedje[1] and Hollocher[7] for the formation of N–N bond in denitrification. The Fe represents the heme d binding site of nitrite reductase; E represents the NO binding site of nitric oxide reductase if such exists in the denitrification pathway.

it is also not clear whether NO occurs as a bound intermediate in the main denitrification pathway and whether there are one or two enzymes responsible for the conversion of NO_2^- to N_2O.

There are two nitrite reductases known to carry out denitrification. One type is a dimer with each identical monomer containing one heme c and one heme d. The heme d has been shown to be the binding site of nitrite. The second denitrifying reductase is less well studied but is known to contain two Cu atoms instead of the heme groups. Both enzymes seem to carry out the same reaction, and there is no reason to assume that there is any difference in the mechanism of N–N bond formation for each type of enzyme.

Averill and Tiedje[1] proposed a mechanism for the conversion of NO_2^- to N_2O that was based on chemically reasonable mechanisms and was consistent with all of the biological evidence (Fig. 6, upper portion)[1]. After the NO_2^- is bound to the heme, it is dehydrated to form a coordinated nitrosyl, a very reactive species. It is proposed that a second NO_2^- undergoes nucleophilic attack on the nitrosyl forming coordinated N_2O_3, the first species with N–N double bond. This species subsequently undergoes two successive two electron reductions through trioxodinitrate, $N_2O_3^{2-}$, and the dehydration to form N_2O. This scheme preserves the two electron per step reduction prevalent in biological reactions and avoids the reactive intermediate products caused by one electron reductions. In this pathway NO is

not an intermediate but it is expected to occur as a side product from the reaction nitrosyl.

A second pathway to N_2O has been proposed by Hollocher[7] which differs in that the reductive steps occur before N–N bond formation (Fig. 6, lower portion). The first step in this pathway are the same as proposed by Averill and Tiedje, but Hollocher now has strong evidence for them. He used a nitronyl trap to show production of the nitrosyl species and showed ^{18}O label exchange between nitrite and water as predicted from the reversible dehydration reaction[5]. After the nitrosyl species he proposes two one electron reductions through coordinated NO to form nitroxyl, NO^-. The nitroxyl dimerizes to form N_2O. In his proposal NO reductase (if one exists) could also contribute to the NO conversion to N_2O through a nitroxyl intermediate. Evidence is not yet conclusive for which of these two mechanisms is responsible for N_2O production. Knowledge of the mechanism could be useful in devising specific inhibitors of denitrification that would not affect other biological processes.

Anaerobic metabolism of organic compounds, especially dechlorination reactions.

Besides denitrification, fermentation is also of practical significance in soils. At the Letcombe Laboratory by Penn and Lynch[15] and associates have shown that acetate produced from decomposition of straw interferes with the establishment of cereal crops; an excellent example of problems that can be caused by fermentation. We have examined the anaerobic metabolism of manufactured chemicals from the perspective that there may be novel reactions carried out by anaerobes that may be useful in pollutant destruction. Most work on the environmental fate of pesticides and xenobiotic compounds has focused on aerobic metabolism. Although there are probably more aerobic biotransformations possible, this does not mean that there are no conversions of potential use. The most interesting reaction that we found is the reductive dehalogenation of the aromatic ring[21]. This is illustrated in Fig. 7 for some of the compounds that we have examined[4,21,23]. In this reaction the aryl chlorine is replaced by a proton. In anaerobic metabolism of chlorinated aromatic compounds the Cl is usually removed after ring cleavage; the latter is often blocked by Cl on the ring. Thus the anaerobic dechlorination represents a mechanism by which the Cl can be removed before ring cleavage thereby making the compound more susceptible to further aerobic or anaerobic degradation.

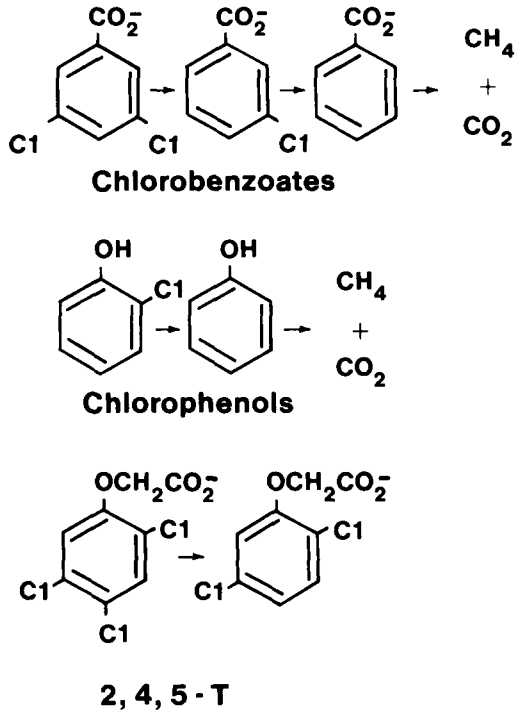

Fig. 7. Examples reductive dechlorination reactions that are carried by anaerobic methanogenic consortia.

We have been unable to enrich a methanogenic consortium from anaerobic sewage sludge using 3-chlorobenzoate as the sole C source[22]. This consortium dehalogenates Cl from the meta position of benzoate and Br from any position[21] and removes the para halogen from 2,4,5-T[23]. This consortium consists of members which dehalogenate the aromatic ring, oxidize benzoate, oxidize butyrate, and produce methane. We have now isolated the dehalogenating organism and find it to be a new, unusual organism both in morphology and physiology. The unique morphological feature is a "skirt" which surrounds the cell.

This unusual organism serves to illustrate the diversity that is probably yet to be discovered among anaerobes. This comment is also supported by the diversity now being recognized among the sulfate-reducing and methane-forming bacteria. Perhaps there is also more diversity of anaerobes in soils than is now recognized. If so, some of these organisms may have potential value. The dehalogenation reactions that we have examined so far have been studied in anaerobic sewage sludge and in lake sediments. Whether they exist in soil is not known, but it would not be surprising since the microbiota of soil

and sediments is not greatly different and anaerobic sludges are now added to many soils.

In summary, soils vary widely in extent and duration of anaerobiosis. Processes carried out by facultative anaerobes, particularly denitrification and fermentation, are the most prevalent and important anaerobic processes in soils. In well drained soils and soils from drier climates, anaerobiosis is of little importance. But in more humid climates or for poorly drained soils, anaerobiosis is of significance. With the new techniques now available a better understanding of the soil anaerobic environment is possible as is a better understanding of the biochemistry, physiology, and diversity of anaerobic organisms.

Acknowledgements The authors work was supported by the U.S. National Science Foundation, U.S. Dept. of Agriculture, U.S. Environmental Protection Agency and the U.S. Air Force.

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