DEVELOPMENT OF A NITROGEN BALANCE IN A LABORATORY SOIL PROFILE WITH A HEAVY APPLICATION OF BEEF CATTLE WASTES

by

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INTRODUCTION

One of the major problems associated with the disposal of large amounts of animal and other wastes on small land areas is the possible pollution of surface and ground waters with inorganic nitrogen compounds. Inorganic nitrogen is released from decomposing organic matter as the result of microbial activity. These compounds are pollutants when they become concentrated in surface or ground-water supplies. The U. S. Public Health Service has set an upper limit of 10 mg/l of nitrate nitrogen for drinking water. Higher concentrations of the nitrate ion in drinking water are considered capable of causing infant Methemoglobonemia.

Traditionally, ground water has been of good quality and municipalities have been able to utilize this valuable source of water with a minimum of concern or treatment. However, if ground water contains large concentrations of nitrate, it must be removed before the water is fit for human consumption. Gunderloy (29) and Barth et al. (8) list several chemical and biological removal processes to do this but these are prohibitively expensive and inefficient.

The prevalence of nitrate polluted water supplies has been studied by several researchers. Stewart et al. (66) took soil samples from 129 sampling points and water samples at many of these points in the Middle South Platte Valley of Colorado.

Samples were taken from under corrals and fields. Many of the water samples had nitrate N concentrations in excess of 10 mg/l. The water from beneath the corrals was not noticeably higher in nitrate concentrations than water from under irrigated fields although nitrate concentrations as high as 5000 pounds per acre in a twenty-foot profile were found under some corrals. This high nitrate level in the soil possesses quite a pollution potential should it be leached into the underlying ground water. Witzel et al. (78) presented the results of a study concerning the effect of farm wastes on the pollution of natural waters. They concluded that: (1) Heavy manure applications can result in dangerously high nitrate concentrations in farm wells, (2) Heavy supplemental irrigation combined with repeated heavy nitrogen fertilizer applications may result in an increase in the nitrates in ground water. Johnston et al. (35) studied the nitrogen levels in tile drainage effluent in the San Joaguin Valley in California. They found that large percentages of the applied N were lost in tile drainage from the silty clay loam soils of western Fresno County.

Law and Bernard (39) indicate that animal wastes, irrigation return flows and excessive fertilizer applications are the major agricultural contributors to the nitrate pollution of our surface and ground-water supplies. Several investigators (32, 35,48,49,50,73) have characterized and evaluated the pollution potential of the various sources.

It is evident that our modern agri-business has become a

water polluter. Nitrate which ends up in our water supplies can emanate from chemical fertilizers or from the oxidation of organic nitrogen compounds. The problem now is to determine the full extent of this nitrate pollution problem and what can and should be done about it.

PURPOSE

The purpose of this project was to study the nitrogen cycle as it occurs in a soil profile with a high loading rate of beef feedlot wastes. A total nitrogen balance was to be run on a soil profile. Equipment was to be developed to control and monitor the environmental factors in the soil. Procedures were to be developed to detect the various nitrogen forms in the soil and to quantify them by determining a nitrogen balance. It was hoped that evaluation of the nitrogen forms in the soil and of the environmental factors affecting their transformations would lead to a better understanding and evaluation of their pollution potential. The prime objective was to contribute to an understanding of the pollution treatment potential of the denitrification process.

LITERATURE REVIEW

Nitrogen Cycle

Nitrogen is an important element in nature. It plays a major role in the life processes of all plants and animals. Its chemical properties are complex because of the many valence states it assumes and the fact that changes in valence can be brought about by living

organisms. Sawyer and McCarty (63) give 3-, 1+, 2+, 3+, 4+, and 5+ as the possible valences of nitrogen.

Some of the important relationships that exist between the various forms of nitrogen in nature are shown in Plate I. This is commonly called the nitrogen cycle. Atmospheric nitrogen, N_2, serves as a reservoir from which nitrogen is constantly removed by nitrogen fixing bacteria as well as the action of electrical discharge. Plant and animal proteins are decomposed to ammonia by bacterial decomposition. Bacteria oxidize ammonia to nitrite and then to nitrate in the process known as nitrification. The resulting nitrate is the primary nitrogen source of higher plants. The process of nitrification can also be reversed to reduce the nitrate nitrogen to nitrite nitrogen. Nitrite can be reduced further either to ammonia or to elemental nitrogen. This latter process is of primary concern where nutrient removal is the desired effect.

In the case of livestock wastes, Plate I illustrates how the nitrogen contained in fecal matter and urine must undergo bacterial decomposition (mineralization) to ammonia and then nitrification before denitrification can take place. Soil scientists and soil microbiologists have investigated nitrogen transformations in soil. Their primary concern has been with conservation of nitrogen as a necessary element for plant growth. Because of this, mineralization and nitrification are well understood and the environmental factors affecting them are fairly well outlined. Mineralization and nitrification will be covered only briefly in this thesis.

Since the major part of the research done on denitrification in soil has been done with an interest in minimizing nitrogen losses from the soil profile, little work has been done on denitrification in soil as a pollution control measure. Literature reviewed relating to denitrification will be covered extensively. The principles discussed can hopefully be applied to denitrification as a pollution control measure.

Mineralization

The conversion of organic nitrogen to the more mobile, inorganic state is known as nitrogen mineralization. The process is the result of microbial utilization of nitrogenous organic molecules. Ammonium is a major product of the reactions. In soils receiving nitrogen rich crop residues, the rate of ammonification ranges from less than 1.0 to 20 ppm nitrogen per day (2). Almost all bacteria, fungi, and actinomycetes play some part in ammonification of organic matter. Different organisms utilize different organic elements as a substrate under varying conditions and, consequently, nitrogen is mineralized even under extreme environmental conditions. The amount of ammonium which accumulates is dependent upon the organism, the substrate, the soil type and other environmental conditions. Bacteria probably dominate in ammonification under neutral or alkaline conditions while fungi become dominant under acid conditions. Anaerobic bacteria dominate when O_2 is lacking. Ammonium production is limited at moisture contents below the permanent wilting percentage but increases with increasing moisture. Alexander (2) indicates the optimum moisture

level is between 50 and 75 per cent of the water holding capacity of the soil, but Wetselaar (74) indicates that ammonification continues at lower moisture levels and that nitrification slows down resulting in an ammonium increase in the soil. Ammonification is also affected by pH. Soil acidity tends to depress the rate. The optimum temperature for ammonification is between 40° and 60°C, indicating a predominance of thermophiles. The above information indicates that added organic nitrogen undergoes fairly rapid mineralization in the soil even under some rather adverse conditions. The soil does, however, maintain a low equilibrium organic nitrogen content.

Immobilization

Immobilization of inorganic nitrogen is another microbial process which occurs in the soil. It probably is of little significance in pollution control but it does bear mentioning as it is a common occurrence in soil. When undecayed crop residues or partially rotted crop residues or manure are incorporated into the soil, the soil microbes utilize and thus immobilize inorganic nitrogen to form new cells and hyphae as they assimilate the added organic matter. Of course the net immobilization of inorganic nitrogen is dependent upon the C:N ratio of the added organic matter and may have a relatively short lived effect. However, nitrogen immobilization is often of significant proportions, agronomically speaking, especially when the C:N ratio of the added organic matter is high. This fact could possibly be of some significance in efforts to utilize the soil profile as a waste treatment system.

Nitrification

Nitrification is the process in which there occurs an increase in the oxidation state of nitrogen. Traditionally nitrification has been considered to be the two step microbial oxidation of reduced nitrogen to nitrate with nitrite as an intermediate. Alexander (1) gives the net reaction as

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

when an ammonium salt is the substrate.

Nitrification is carried on primarily by autotrophic bacteria. Alexander (2) lists the following seven genera.

Ammonium Oxidizers	Nitrite Oxidizers
Nitrosomonas	Nitrobacter
Nitrosococcus	Nitrocystis
Nitrosospira	
Nitrosocystis	
Nitrosogloea	

Only Nitrosomonas and Nitrobacter are normally abundant in soil and are undoubtedly the major nitrifying chemoautotrophs. They are nearly always found together in the soil.

Some heterotrophic microorganisms have been found to carry on nitrification. Nitrification is not, however, obligately associated with the development of the heterotrophs. The extent of the role which these organisms play in nitrite and nitrate formation is difficult to assess. In general they are inefficient nitrifiers, but due to their large numbers they could still make a small contribution in the total nitrification process.

The rate of nitrification in soils is highly variable depending upon several interrelated environmental factors. These factors include soil pH, oxygen level in the soil, moisture level, soil type, soil temperature, level of organic matter, depth, nutrient supply and the presence or absence of certain microbial inhibitors.

Soil pH has a governing effect upon the rate of nitrification. Since autotrophic bacteria are the primary nitrifiers, nitrification occurs slowly or not at all under acid conditions. Bacteria do not tolerate highly acid conditions well. The optimum pH for Nitrosomonas falls in the range from 7 to 9 but activity is found outside of this range (1). The optimum for Nitrobacter also lies in the neutral to slightly alkaline range with activity detectable between approximately pH 5 and 10.

Nitrifying autotrophs are obligate aerobes and most all of the heterotrophs are aerobic as well. The oxygen content of the soil is one of the primary determinants of the nitrification rate in soil. The aeration of the soil is determined by a number of factors including soil structure, soil moisture level, temperature variations, and the activity of the soil biota. The soil structure and soil moisture level combine as a primary determinant of nitrification rate. The optimum moisture level generally lies between one-half to two-thirds of the water holding capacity of the soil. Temperature changes increase aeration due to changing gas volumes. The respiratory gases of the soil biota as well as the burrowing action of some of the larger species also contribute to the aeration of the soil.

Depth is an indirect determinant of nitrification rate. At shallow depths, the moisture level is too low to support active microbial action except immediately following precipitation. At greater depths the oxygen concentration is low and the nutrient level is low so a large nitrifying population can not build up. The optimum temperature for the transformations lies between 30° and 35°C. The upper soil layers would therefore be more favorable from this standpoint. Nitrification rate varies with season. The highest rates of activity generally occur during spring and fall with very low activity occuring during the cold of winter and the dry conditions of summer.

The nutrient level of the soil is a primary factor affecting nitrogen transformations. Since nitrification in soil is primarily an autotrophic process, the energy substrate is probably the nutrient which limits activity. The quantity of ammonium-nitrogen readily available to microorganisms in the soil is rarely appreciable. Therefore, the oxidation of ammonium occurs more rapidly than ammonium formation.

The organic matter in the soil has several possible effects on the nitrifiers. It can serve as a substrate for ammonium formation, thus providing oxidizable substrate or it may contain compounds which inhibit bacterial development. The microbial breakdown of organic matter may require large amounts of nitrogen for cell synthesis, thus immobilizing more nitrogen and limiting nitrate accumulation.

The presence or absence of microbial inhibitors in the soil can have desirable or undesirable effects. There are many compounds

which can be toxic to the nitrifiers under the right conditions. Even nitrite can be toxic under some conditions. These toxic compounds are not all well known and will not be discussed further here.

Nitrification has many variables which affect it and these make it difficult to quantify and predict. However, Sabey (61) presented a formula which he used to predict nitrate accumulation in soils under varying conditions of temperature and soil moisture tension. McLaren (44,45) has developed mathematical models of nitrification in a steady state process. Some of the factors in his equations still need to be evaluated over the range of conditions found in field situations.

Denitrification

In nitrogen balance experiments it is commonly found that there is a loss of nitrogen from the system which cannot be accounted for by residual nitrogen, leaching losses, or nitrogen removal by crops. Allison (5) documented a large number of lysimeter experiments in which the nitrogen deficit averaged 15%. This loss presumably occurs by ammonia volatilization or by the reduction of nitrate to a gaseous form.

Denitrification generally refers to the biological reduction of nitrate and nitrite to volatile gases. These gases usually are nitrous oxide and/or molecular nitrogen. Recent research has shown that nitrogen losses in soil occur by a combination of chemical and biological reactions (17). In this thesis, the term denitrification implies the gaseous loss of nitrogen by either biological or chemical mechanisms excluding ammonia volatilization.

When it became apparent that denitrification resulted in large enough nitrogen losses to be of economic significance, much research was initiated to evaluate the pathways of denitrification and the factors affecting the reaction rate. Alexander (2) lists four proposed reactions for nitrogen volatilization: (a) non-biological losses of ammonia; (b) chemical decomposition of nitrite under acid conditions to yield nitrogen oxides; (c) production of N_2 by the non-enzymatic reactions of nitrous acid with ammonium or amino acids; and (d) microbial denitrification leading to the liberation of N_2 and N_2O.

Although ammonia volatilization is not one of the processes of denitrification, it will be discussed here briefly as it could aid in the removal of excess nitrogen from the soil and thus help minimize nitrate pollution of our water supplies. The volatilization of ammonia is appreciable under conditions of high pH and warm temperatures. As much as one-fourth of the ammonium supplied in fertilizers or formed microbiologically may be lost in the gaseous form (2). Gaseous ammonia loss from surface-applied manure or urea will take place even at sites in which the underlying soil is highly acid if the increase in alkalinity associated with ammonification is sufficiently great.

In acid environments, pH below about 5.5, nitrite decomposes spontaneously to nitric oxide, NO (2). Broadbent and Clark (17) depict the reaction as follows:

$$3HNO_2 \rightarrow 2NO + HNO_3 + H_2O \qquad (1)$$

The higher the acidity, the greater the magnitude of the reaction. Several investigators have found that nitric oxide is evolved

under highly acid conditions (4,58,68), but Cady and Bartholomew (19) indicate that the above equation does not indicate all of the products which are present and active in influencing the reaction rates. Due to the inability of the nitrifiers to exist actively under low pH values, the chemical denitrification of nitrite is usually limited by a lack of nitrification. The rate of nitrogen loss has been shown to be quite low under high acidity (65) and consequently this type of loss is rarely of significance in conditions which exist under field conditions. The most likely condition for this denitrification pathway to become significant would be where large applications of nitrate fertilizer are added to an acid soil or where the buffering capacity of the soil is so low that the pH falls drastically during nitrification.

Under suitable conditions nitrous acid may react with amino acids to yield molecular nitrogen according to the following equation:

$$RNH_2 + HNO_2 \rightarrow ROH + H_2O + N_2 \qquad (2)$$

The possible role of this reaction in soil has been studied by several workers (4,5,6,26,65). This reaction has the same major drawback as the one discussed above in that it occurs only under highly acid conditions, namely pH below 5. For this reason it assumes little importance in the total denitrification picture.

In a reaction quite similar to the above equation, ammonia may react with nitrous acid to yield molecular nitrogen.

$$NH_3 + HNO_2 \rightarrow N_2 + 2H_2O \qquad (3)$$

Considerable research has been devoted to this reaction (4,26).

Compounds such as ammonia, urea, methylamine, purines and pyrimidines react with nitrous acid though not as readily as do the amino acids (17). Smith and Clark (65) observed that nitrous acid and ammonium sulfate reacted much slower than did nitrous acid and alanine. Allison (4) suggests that gaseous losses of nitrogen from soils may be greater by ammonium nitrite decomposition than by either of the previously discussed pathways. He further suggests that much of the nitrogen loss attributed to so called aerobic denitrification is actually due to the formation and decomposition of ammonium nitrite. Smith and Clark (65) presented evidence against this occurrence. They found that the reaction between ammonium and nitrite ions was not nearly as important as the reduction of nitrite to nitrogen gas by some component of the soil complex. Evolution of N_2 and N_2O and diminution of nitrite content without concomitant diminution of substrate ammonium was observed. The presence of soil interferred with the ammonium-nitrite reaction. Thus it seems doubtful that the reaction represented by formula number 3 contributes to chemical denitrification in soil.

Broadbent and Clark (17) discuss further reactions of nitrous acid with various soil constituents. They indicate several reactions which could possibly explain the extent to which chemical denitrification seems to occur under some conditions. These pathways need further research for an adequate evaluation.

The major mechanism of nitrogen volatilization is microbial denitrification. The process is accomplished by facultative anaerobic bacteria capable of using nitrate in place of oxygen as a hydrogen acceptor. N_2 and N_2O are the common gaseous products

of the process. Elemental nitrogen is usually the dominant product, but N_2O is evolved under some conditions as well. The process normally occurs only when the partial pressure of oxygen is low. The steps and intermediates of the process have not been definitely and clearly determined. Many environmental variables affect the process and much research has been done to identify and quantify these.

The size and activity of the denitrifying population in different ecological circumstances is a key factor in determining the rate of loss of nitrogen from soils. The microorganisms responsible for denitrification are classified in the class of Schizomycetes (69). Pseudomonas, Achromobacter, Bacillus and Micrococcus as well as Thiobacillus denitrificans and an occasional Chromobacterium, Mycoplana, Serratio, or Vibrio species may catalyze the reduction (2). Pseudomonas and Achromobacter are the dominant denitrifying genera in soil.

The possible actions of the denitrifiers on nitrate include: (a) complete reduction of ammonium frequently with the transitory appearance of nitrite; (b) an incomplete reduction and an accumulation of nitrite in the medium; and (c) a reduction to nitrite followed by the evolution of gaseous compounds, i.e., denitrification. Plate II shows the denitrification process as suggested by Nommik (52) with nitramide as the possible unknown intermediate in the process. Several investigators have studied the products of denitrification and tried to discover the exact reactions including all of the intermediates (3,22,52,62,72). Several schemes

have been proposed to show a series of intermediates between nitrate and nitrogen gas (17). Some of the proposed intermediates hyponitrite, nitramid, and imido-nitric acid, have not been identified in denitrifying systems. There is, however, fairly good agreement that the reduction sequence includes nitrate, nitrite, nitrous oxide, and nitrogen, in that order. Cady and Bartholomew (20) determined this sequence by incubating soil samples containing tagged nitrate in a closed glass apparatus equipped to provide internal gas circulation. They found the initial gaseous product to be small amounts of nitric oxide (NO). They contributed this to the decomposition of nitrous acid. Nitrous oxide then appeared and as it increased, nitric oxide decreased. The final product to appear was elemental nitrogen. It increased in amount sufficient to account for 83 to 95% of the tagged nitrate added to each soil sample. Cooper and Smith (22) found the sequence of $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$ in all seven soils which they incubated in a closed anaerobic system in which gas analysis was done by gas chromatography.

The position of nitrous oxide in the main pathway of denitrification has not been unequivocally determined. Wijler and Delwiche (76) found nitrous oxide to be the major product of denitrification under most soil conditions. The relative proportion of N_2O and N_2 was dependent upon pH however. Allen and Van Niel (3) concluded that nitrous oxide was not the precursor of elemental nitrogen in cultures of Pseudomonas stutzeri. Sacks and Barker (62) working with Pseudomonas denitrificans presented evidence indicating that nitrous oxide cannot be an obligatory

precursor to the formation of molecular nitrogen. Broadbent and Clark (17) indicate agreement with Nommik's (52) conclusion that nitrous oxide is an obligatory precursor of molecular nitrogen. In view of some of the evidence presented above, it seems that this cannot be correct. One possible solution to this controversy was presented by Alexander (2) and is shown here in Plate II.

As indicated by some researchers (2,72,76), the denitrification process can reduce nitrate to ammonia, the original substrate for nitrification. Alexander (2) and Verhoeven (72) indicate that the extent to which this occurs is small indeed. Wijler and Delwiche (76) indicate that nitrate reduction to ammonia was only extensive at extremely high carbon substrate levels under anaerobic conditions. Broadbent and Stojanovic (18) studied the effect of partial pressure of oxygen on denitrification and found that the reduction of nitrate to ammonia was almost negligible at all oxygen concentrations studied.

Aside from the presence or absence of a bacterial population capable of denitrification, the most important determinant of the rate of nitrate reduction is the influence exerted by the environment. The effect of various environmental factors on denitrification are indicated by several researchers (2,16,17,18,21,23,24, 27,36,40,41,42,52,53,54,68,69,72,75,76).

The aeration of the soil is a primary determinant of the partial pressure of oxygen in the soil. The rate of denitrification is highly dependent upon the partial pressure of oxygen in the soil since nitrate reduction is a facultative property (72). The bacteria

responsible proliferate as favorably under aerobic conditions as under anaerobic conditions and under excessive aeration only nitrate assimilation takes place. Only when the oxygen supply is very limited will the denitrifiers utilize nitrate as a hydrogen acceptor. There have been seemingly conflicting reports indicating that denitrification has taken place under seemingly adequate O_2 tension to inhibit such reduction (2,17,18). There probably is no real contradiction in the results indicating bacterial denitrification under aerobic conditions. The problem probably lies in the use of the terms aerobic and anaerobic. The fact that denitrification has been shown to occur in soils maintained at normal O_2 tensions merely demonstrates that many pores and interstices in the soil are never entirely oxygenated. Several factors affect the degree to which the oxygen tension is lowered. The prevalence of very small or very large soil particles decreases the oxygen diffusion rate into the soil pores (52). The biological oxygen demand may be high due to the rapid decomposition of an energy substrate. The rate of oxygen diffusion may become limiting and the micro-environment may become anaerobic. Small pores filled with water are especially subject to the development of anaerobic conditions even though larger pores surrounding the area may be filled with air.

The moisture level in the soil affects denitrification mainly from the standpoint of its affect upon the O_2 diffusion rate into the soil. Denitrification is almost completely stopped at moisture levels below 60% of the water holding capacity of the

soil and is fairly slow even at 80% (2,17). Nitrogen losses are the highest under water logged conditions and continue to increase up to 450% of the water holding capacity of the soil (16). The effect of moisture level upon denitrification has been confirmed by others as well (24,52).

The organic matter level in the soil plays a dual role with respect to denitrification. Organic matter acts as an energy substrate for the bacteria, serving as a hydrogen donor for denitrification, and its decomposition increases the oxygen demand in the soil. Denitrification is enhanced by high levels of easily decomposable organic matter in the soil. Bremner and Shaw (15) found that denitrification was not detectable when soils of low organic matter content were incubated with nitrate under water logged conditions, but it was readily detectable when glucose or straw was added or when soils of higher organic matter content were used. In a series of tests using insoluble materials the rate of denitrification varied with the resistance of the materials to decomposition, being most rapid with cellulose and least rapid with lignin and sawdust. In another report (16) they showed that the rate of denitrification with leached straw or straw previously decomposed in soil was much slower than with new straw. This suggests that it is the water soluble and readily decomposable constituents of straw which are the most effective in promoting denitrification. Other research indicates the effects of organic matter in the soil upon heterotrophic denitrification (23,42,52,54). Broadbent and Clark (17) indicate that

the rhizosphere of living roots is a site of some interest with respect to denitrification. The rhizosphere organisms consume much oxygen during the breakdown of root excretions, thus reducing the oxygen tension in the soil. The root excretions may also serve as hydrogen donors in the reactions.

The pH of the soil is another of the interrelated environmental factors affecting denitrification. The denitrifying bacteria are sensitive to pH and rarely exist in high numbers under very acid conditions. Denitrification occurs very slowly in acid soils and very rapidly in soils of high pH (16). Wijler and Delwiche (76) and Nommik (52) agree that the denitrification rate is practically constant above pH 6. They also found that the denitrification gas was chiefly nitrous oxide at low pH values. The reduction of N_2O is inhibited below pH 7, but Cady and Bartholomew (20), working with an acid soil, observed essentially complete reduction of nitrous oxide to molecular nitrogen. It should be noted that denitrification not only is affected by pH but affects it. Hiltbold and Adams (33) worked with the soil acidity changes due to applied nitrogen. They found that the pH of the soil was increased by denitrification regardless of the probable gaseous form of loss. This helps to correct for the drop in pH which results from nitrification.

The denitrification rate seems to be independent of nitrate concentration over a fairly wide range (22,52). The relative proportion of N_2O in the denitrification gases is higher at high nitrate concentrations. Of course denitrification is limited by low nitrate levels.

Denitrification is highly temperature dependent. The reaction will proceed slowly down to as low as 2°C. The optimum temperature is at 25° and above (16), but the transformation is rapid even at higher temperatures. The reactions will proceed to 60° or 65°C but not at 70°C.

In summary denitrification is clearly important as a means of nitrogen removal from the soil. The exact path and significance of all of the reactions are not definitely known. A good supply of readily oxidizable organic compounds, high nitrate levels and poor drainage favor the reactions. A near neutral or basic pH as well as a fairly high temperature also contribute to rapid denitrification. The major cause of nitrogen volatilization is the heterotrophic denitrifying bacteria, but chemical volatilization becomes significant under some conditions.

Nitrogen Removal by Denitrification

The ability of soil to purify sewage effluent or similar low quality water has prompted considerable interest and research in this area. Several municipalities have been experimenting with soil disposal of municipal sewage effluents by applying the effluents for irrigation of crops (25,77). Effluent applied in excess of the water needs of the crops soon reaches the groundwater, but in the process of percolation it undergoes a great improvement in quality. Unfortunately the effect of the effluent on the soil structure and chemical properties of the soil is not always good and the degree of treatment is not always satisfactory.

The removal of excessive nitrogen in wastes applied to land

poses a big problem. Complete aerobic mineralization of added organic nitrogen occurs near the soil surface where inundation by liquid wastes occurs periodically (11,37). The resulting ammonia is subject to adsorption to the soil or to bacterial nitrification. Bouwer (11), and Koelliker and Miner (37) found that nearly all ammonia was nitrified in the soil profile if sufficient dryup times were allowed between liquid effluent applications to establish good aeration in the soil profile. Once the nitrogen is in the nitrate or nitrite form, denitrification may occur if the organic matter concentration is high enough and the oxygen tension is low enough, assuming pH, temperature and microbial populations are satisfactory. Koelliker and Miner (37) found a reduction of approximately 80% in the nitrate and nitrite forms of nitrogen which developed in anaerobic lagoon effluent percolating through a 48 inch soil profile. They attributed this loss to denitrification. They noted a loss of organic matter also and indicated that the loss of organic matter in the soil corresponded to organic nitrogen loss. This indicated that the present practice of removing organic matter from effluent before applying it to the soil is probably detrimental from the standpoint of reducing nitrogen pollution by denitrification.

One factor which increases the prevalence of high nitrate concentrations in ground water where large amounts of organic nitrogen are applied on the soil above is the high mobility of the nitrate and nitrite ions in the soil solution. Unlike ammonium, these ions are not adsorbed readily onto the soil particles. Preul (57) found that concentrations of 60 mg/l ammonium nitrogen

had decreased to 3 to 4 mg/l after the soil solution had traveled 20 feet in a fairly sandy soil. Nitrates on the other hand remained very high in concentration even after the soil solution had traveled over 100 feet through the soil.

Robeck et al. (60) reported extensive work on determining design factors for soil systems to treat wastes. They worked with the treatment characteristics of sand columns with septic tank effluent percolated through them. In this system nitrification was found to occur at a much higher rate when percolated through a deep bed. The presence of the water table close to the surface limited the oxygen availability for nitrification. There were no figures reported for nitrogen loss by denitrification.

In summary, little work has been done on denitrification in soil as a pollution control measure. The agronomists who have worked on denitrification in soil have been concerned with nitrogen conservation. In many cases where sewage is applied as irrigation water, the nitrogen added is desirable as a fertilizer element. In many other places where organic nitrogen has become concentrated on or near the soil surface, it eventually finds its way to the underlying ground water in such high concentrations, and usually in the nitrate form, as to constitute a dangerous water pollutant. Several workers (11,37,70,73) indicate the occurence of denitrification where organic wastes have been applied to the soil for treatment. A high degree of nitrification is a necessary prerequisite to denitrification. This is favored by long enough dry-up periods to prevent septic conditions in the soil. Denitrification requires a high microbial level which in turn is dependent

upon organic matter level. A low oxygen tension and a neutral or basic soil pH benefit the reactions. The oxygen tension is dependent upon the moisture level and the soil particle size distribution, as well as the organic matter level. The other important determinant of denitrification is temperature. This is largely dependent upon season and denitrification is slowed during late fall and winter. It has been indicated by several researchers that the variables are favorable enough at least part of the time for a very significant portion of the organic nitrogen applied to the soil to undergo denitrification and leave the soil system as a gas.

Methods of Studying Denitrification

Over the years many different methods of studying denitrification have been devised. Lysimeter studies are done on a total soil profile and are usually operated to simulate natural conditions as nearly as possible. Another approach which is widely used is the incubation of a small soil sample in an enclosed glass apparatus so that all environmental variables can be controlled. The other commonly used approach is that of percolating a nutrient solution through a small sample in an apparatus providing control of environmental conditions. Apparatus used to control environmental variables ranges from practically none for the lysimeters to very elaborate for some incubation experiments.

Allison (5) lists numerous lysimeter experiments in which efforts were made to determine a nitrogen balance. These experiments all have the same general drawback in that they are unable to account directly for nitrogen gains due to fixation and

losses due to denitrification. They are able only to give an unaccounted for loss of nitrogen and assume that it was due to nitrogen volatilization. Overrein (53) attempted to solve this problem with gas sampling hoods and applications of N^{15} as a tracer.

There have been many experiments on denitrification using some sort of incubation flasks. This is the approach taken by many researchers when it is desired to study the effects of the different environmental variables (3,6,16,18,19,20,21,22,26,27, 33,40,42,43,52,61,62,65,71,74,76). Temperature is of course easy to control but the aeration and moisture tension in these experiments is sometimes controlled by elaborate equipment of different types. Temperature effects will be discussed briefly later in this thesis. The amount of oxygen utilized and the amount of CO_2 produced usually are monitored as indications of the amount of microbial activity. The gases given off usually are trapped and analyzed to indicate the pathway and amount of denitrification taking place. This approach is well suited to the study of the effects of individual variables, but its one drawback is that it does not indicate the relationship of all variables when combined in a soil profile.

The percolation of a nutrient solution through a soil has been utilized by several investigators (28,44,45,75), but it has not been nearly as useful as incubation techniques. McLaren (44, 45) has exploited this approach to determine a mathematical model to describe denitrification under steady state percolation of a nutrient solution.

One of the main problems in trying to create natural conditions in a soil incubated in the laboratory is the monitoring and controlling of the soil moisture tension. Bartholomew and Broadbent (9) explain one apparatus to do this but its use probably is limited to incubation experiments. Richards and Ogata (59) describe a thermocouple for the measurement of vapor pressure in soil systems at high humidity. Such an instrument could prove very useful if sufficient instrumentation is available for its use. Another indication of moisture level and aeration in the soil is redox potential (17,54). Measurements of this quantity can be obtained by means of a pH meter with the proper electrodes. A simple instrument for the measurement of soil moisture tension is the tensiometer (64). Its use in denitrification studies has not been mentioned in any of the research reviewed. This possibly is due to its limited range but it should read soil moisture levels down to 50% of the water holding capacity even in clay soils (64). This should cover the moisture range under which denitrification normally occurs.

The most critical problem in trying to run quantitative studies on denitrification probably is the analysis of the various gases evolved. There are several approaches to the problem. One of these methods entails the use of N^{15} as a tracer in the nitrogen substrate furnished to the denitrifiers (12,13,31). The gases evolved can be analyzed by the use of a mass spectrometer. Mass spectrometry has been useful in determining which gases are given off and in what amounts. Hauck and Melsted (31) also used the infra-red spectrophotometer to analyze for gaseous nitrous oxide.

The other widely used method of gas analysis, gas chromotography, has been used by several investigators (10,38,58,65,67). These researchers indicate a number of methods by which all of the common gases concerned in soil denitrification can be quantified using the gas chromatograph. The gases of interest in denitrification which may be detected by proper techniques of gas chromatography include N, N_2O, NO, NO_2, NH_3, O_2, and CO_2. Smith and Clark (65) describe equipment such that all of these gases may be analyzed by a single instrument.

In order to evaluate nitrogen transformations in the soil, a way of quantifying the various forms of nitrogen is needed. The literature was not covered extensively with respect to quantitative analysis of soils as this was not deemed necessary. Suffice to say that the method of Bremner and Keeney (14) for determination of various forms of nitrogen in the soil appears to be quite good and that methods for determination of nitrogen forms in the soil solution are given in other references (7,30). There are also other quantitative procedures known for the determination of nitrogen quantities.

In summary, there are many known methods of studying nitrification and denitrification. Many of these are very good, but there are few methods developed to determine a direct nitrogen balance on a total soil profile. Many facts are known about the nitrogen cycle but there is still much that could be learned from equipment capable of running a total nitrogen balance on a soil profile. This is especially true with respect to studies of denitrification as a preventive of nitrate pollution of ground water.

PROCEDURES AND EQUIPMENT

In order to study denitrification under as natural conditions as possible, it was desired to design laboratory equipment which would allow a whole soil profile to be studied. In order to determine a total nitrogen balance, it was necessary to incubate the soil profile in a closed container so that all products of denitrification could be quantified. The mobility of the various nitrogen forms in the soil is also of interest so it was desirable to be able to trace their movement through the soil. Thus it is evident that an apparatus which combined the total soil profile of a lysimeter, the closed gas collection system of an incubation apparatus and the soil solution sampling ability of a soil percolation apparatus would be required.

The apparatus which was designed to meet the above listed needs is shown in Plate III. The soil profile is contained in a plexiglas column with an air inlet at the top and with 12 water sampling positions down the side and one water outlet at the bottom.

Water samples were taken from the soil at 4-inch depth intervals. The samplers were placed as shown in Plate III so that there is a one foot vertical distance between samplers in each of three vertical sampling planes. The water samples were drawn out of the soil through fritted glass samplers inserted into the soil at each sampling position. A perforated metal plate was supported about 1 inch off of the bottom of the column. A 3-inch layer of course sand above this plate supported the soil profile. The soil solution drawn out of the soil column at the sampling positions was

trapped in 50 ml Erlenmeyer flasks mounted down the side of the column. A 2-liter bottle was used to trap the soil solution drawn out of the bottom of the column. Any gas drawn from the soil column was passed through a 0.1 N solution of sulfuric acid to scrub out any ammonia volatilized and through a 0.2 N solution of sodium hydroxide to scrub out any CO_2 produced. The gases then passed through the gas chromatograph for analysis and through a wet test volume meter.

The gas and water samples were drawn from the column by a tubing vacuum pump. The suction line from each of the 13 collection flasks ran through a solenoid operated pinch clamp to a common suction line, which led through the vacuum pump, the two scrubber bottles, the gas chromatograph sampling loop and the exiting wet test gas volume meter.

An automatic sequence control panel developed by Mensch and Reece (46) was utilized to control the vacuum pump and the solenoid valves, opening and closing the suction lines to each sample bottle. The control panel allowed a great degree of flexibility in controlling what sampling positions were being sampled, for how long, and how long the time lag, if any, between sampling at each position in the soil column. All samplers or any combination of samplers could be operated simultaneously. The vacuum pump operated only when one or more of the solenoids was open. The control panel would repeat any pre-set sampling sequence continuously but a sensing system was devised to drop sampling bottles from the sequence when they became full. This was accomplished by inserting

2 wire leads into the top of each bottle and applying a 115 volt potential across these wires. When the fluid level in the bottles reached that of the wires, the fluid completed a circuit which activated a 24 volt relay. The relay activated the stepping circuit in the control panel which skipped to the next step in the sampling sequence. Therefore, each time the sampling sequence was repeated, any full sampling bottles were skipped.

The gas chromatograph was a Fisher-Hamilton Model 29 with a strip chart recorder. It was controlled by the electrical circuit shown in Plate V. The circuit was designed such that for each one-hundredth cubic foot of gas drawn from the column, the needle on the gas meter would make contact with one of the 10 points around the circumference of the meter face. This would activate the 24 volt relay which energizes the sampling solenoid and the recorder. The sampling solenoid connects the .25 ml sample loop with the carrier gas stream. After 5 seconds, a time delay tube turns the solenoid off and the sampling valve returns to the original position. The strip chart recorder runs until the gas sample is analyzed after which the reset timer turns it off. The instrument is then ready to take a new sample after another one-hundredth cubic foot of gas has been drawn from the column.

The gas chromatograph was equipped with two separation columns. The first column is 6 foot long by 1/4 inch in diameter and was packed with Di-2-ethylhexylsebacate on 60-80 mesh Columpak. The second column is 1/2 foot long by 3/16 inch in diameter and is packed with 42-60 molecular sieve. Samples of nitrous oxide,

nitrogen gas, and normal air were used as standards to calibrate the chromatograph. The analysis of gas samples was printed out by the strip chart recorder. Gas concentrations of each sample analyzed were determined by substituting the known values in the following equation:

$$C_s = \frac{H_s}{H_{std}} (C_{std})$$

Where: C_s = Concentration of sample component, % by volume.

C_{std} = Concentration of standard component, % by volume.

H_s = Peak height of sample component, in chart divisions.

H_{std} = Peak height of standard component, in chart divisions.

The soil column was constructed of 3/8 inch plexiglass plate. Inside dimensions in cross section were 4 by 9-1/2 inches so that a Troxler model 1379 2-probe depth density gauge could be used to determine the moisture level in the soil periodically. The broadest external dimension of the column in cross section was 10-1/4 inches which was just narrow enough that the column would fit between the 2 probes of the density gauge. The manufacturers of the instrument indicated that incremental measurements of density changes due to moisture or compaction may be made to within less than 0.1 pounds per cubic foot. This indicated that if changes in soil density due to compaction were eliminated, changes in density due to change in moisture level could be measured to within 0.1 pound per cubic foot. A change in density

of 0.1 pound per cubic foot can be caused by a small change in moisture level so it was hoped that the density gauge could be used to determine changes of a few percent in moisture level in the soil.

The soil used in the soil column was a loamy sand. It was sieved through a course screen to remove any large sticks or stones. The soil was maintained in the moist condition in which it was taken from the field. A weighed amount of the soil was added to the soil column and compacted by tapping the side of the column. The soil was further compacted by cyclic wetting and drying. This was accomplished by adding water to the soil surface and then drawing it out of the bottom of the column by applying a vacuum. The vacuum was maintained on the soil column for a period of time after the water was recovered at the bottom so that air was drawn through the soil to dry it. Cyclic wetting and drying of the soil column was continued for 10 days after which it was assumed that changes in density due to compaction were negligible. The soil was saturated with water from below, and the density gauge was used to determine the saturated density of the soil at 3 specific positions in the soil profile. The soil profile was then dried out one more time. Another density measurement was taken to represent approximately 0% saturation. The soil saturation level should then be a direct percentage of the difference between the soil density at saturation and at 0% saturation.

Unfortunately, 7 weeks of experimentation and research with the density probe showed that it could not be utilized to determine

the moisture level in the soil. It was impossible to calibrate the instrument with any reproducibility. The calibration also tended to drift for no apparent reason. A representative of the manufacturer of the instrument was neither able to offer a reason for the difficulties nor a solution to them.

A layer of air dry manure sufficient to equal an application rate of approximately 50 tons per acre was applied to the soil surface. This was covered by about 4 inches of soil. The top of the column was sealed and the samplers were inserted into the sides of the soil profile. Small soil cores were removed from the side of the soil profile to make room for the fritted glass samplers in the soil. These soil cores were saved and analyzed for nitrogen forms present in the soil so that the amount and forms of nitrogen in the soil was known prior to the start of tests.

On July 22, the first of 13 week long sample collecting runs was started. The sampling bottles were connected to the column and 2 liters of water was added to the soil surface. The control panel was energized and water samples were drawn as the water percolated through the soil. Several sampling sequence times were tried during the 13 runs and it became evident that the sampling controls were good enough that sufficient water samples could be drawn from the soil without drawing excessive air into the soil. Thus it was indicated that water samples could be taken from the soil without unduly upsetting the soil processes and equilibria if a system could be developed to monitor

these soil processes. After experimenting on the first several runs, the sampling sequence was set so that each of the 12 samplers sampled for one minute with a three minute off period before the next sampler came on. The bottom bottle was on for 10 seconds during each sampling cycle until the 12 small sample bottles were filled. It normally took less than 8 hours for the small samplers to fill. After they were full, the hoses to them were pinched shut with pinch clamps and the solenoids were turned off. The bottom sampler was then the only one left on and it sampled once every 36-minute cycle as the control panel by-passed the one minute sampling periods but counted the 3-minute off periods. The bottom sampler continued to operate for the remainder of the week for each of the 13 sampling runs. After the first 6 runs, the sampling time was always lengthened to 90 seconds per cycle after the small samplers were full as it became apparent that anaerobic conditions had developed in the soil during these runs.

The water samples taken out of the column were preserved by adding sufficient sulfuric acid to each sample bottle to retard microbial activity prior to connecting the sample bottles to the column. These samples were taken into the laboratory each week and analyzed for nitrate, nitrite and ammonia. Nitrate was determined by the Brucine method (7). Nitrite was determined colorimetrically using the method of the Hach Chemical Company (30). This method was chosen over that of Standard Methods (7) because it was simpler and the amount of nitrite found was expected to be

small compared to the other forms of nitrogen in the soil and, thus, not as important in determining a total nitrogen balance. Ammonia was normally determined by direct nesslerization (7) but the distillation procedure with titration of boric acid was necessary in runs 3, 4, and 5 due to interferences. These interferences were the result of anaerobic microbial processes. The distillation procedure was much more time consuming than direct nesslerization and the results did not appear to be as precise.

Following the 13 runs, the soil was removed from the soil column and samples were taken to determine the amount and forms of nitrogen left in the soil. Samples were taken above the manure layer, at the manure layer, and at each of the 12 water sampling positions. These samples, as well as the sample of soil taken before the testing started, were analyzed for nitrate, ammonia, organic nitrogen, organic matter, and pH by the KSU Soil Testing Lab. Nitrate and ammonia were determined by steam distillation (14). Organic nitrogen was determined by micro-kjeldahl and organic matter was determined by a wet combustion method. Nitrite was not determined as it was believed to be negligible.

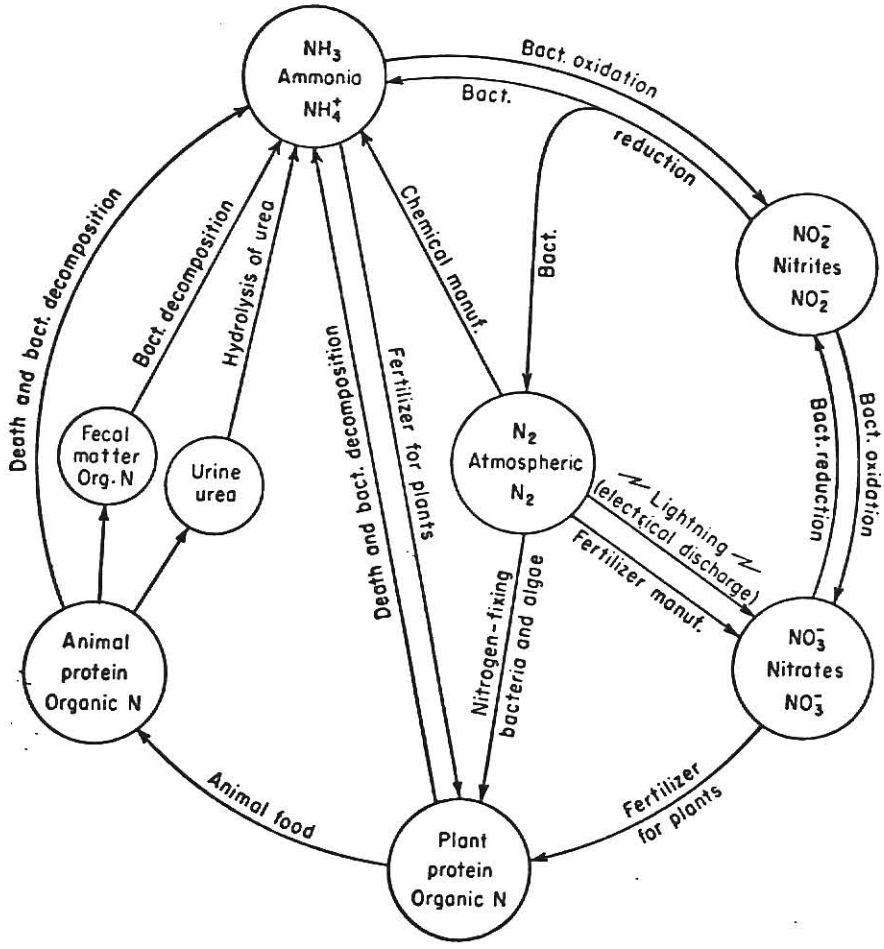
The data obtained during the 13 test runs along with the data taken before and after the tests on the amounts and forms of nitrogen in the soil were used to determine, if possible, a total nitrogen balance on the soil column. The gas data and the water sample data were used in an attempt to determine what reactions occur in the soil and at what location. The various environmental factors are also of interest with respect to the reactions occuring in the soil profile.

ILLUSTRATIONS

EXPLANATION OF PLATE I

Nitrogen Cycle (63)

PLATE I


NH_3
Ammonia
NH_4^+
Bact. oxidation
Bact. reduction
NO_2^-
Nitrites
NO_2^-
NO_3^-
Nitrates
NO_3^-
N_2
Atmospheric
N_2
Lightning (electrical discharge)
Fertilizer manuf.
Chemical manuf.
Bact.
Bact. reduction
Bact. oxidation
Death and bact. decomposition
Bact. decomposition
Hydrolysis of urea
Fertilizer for plants
Death and bact. decomposition
Nitrogen-fixing bacteria and algae
Fecal matter Org. N
Urine urea
Animal protein Organic N
Plant protein Organic N
Animal food
Fertilizer for plants

EXPLANATION OF PLATE II

Possible Pathways of Denitrification

Fig. 1. Nommik (52)

Fig. 2. Alexander (2)

PLATE II

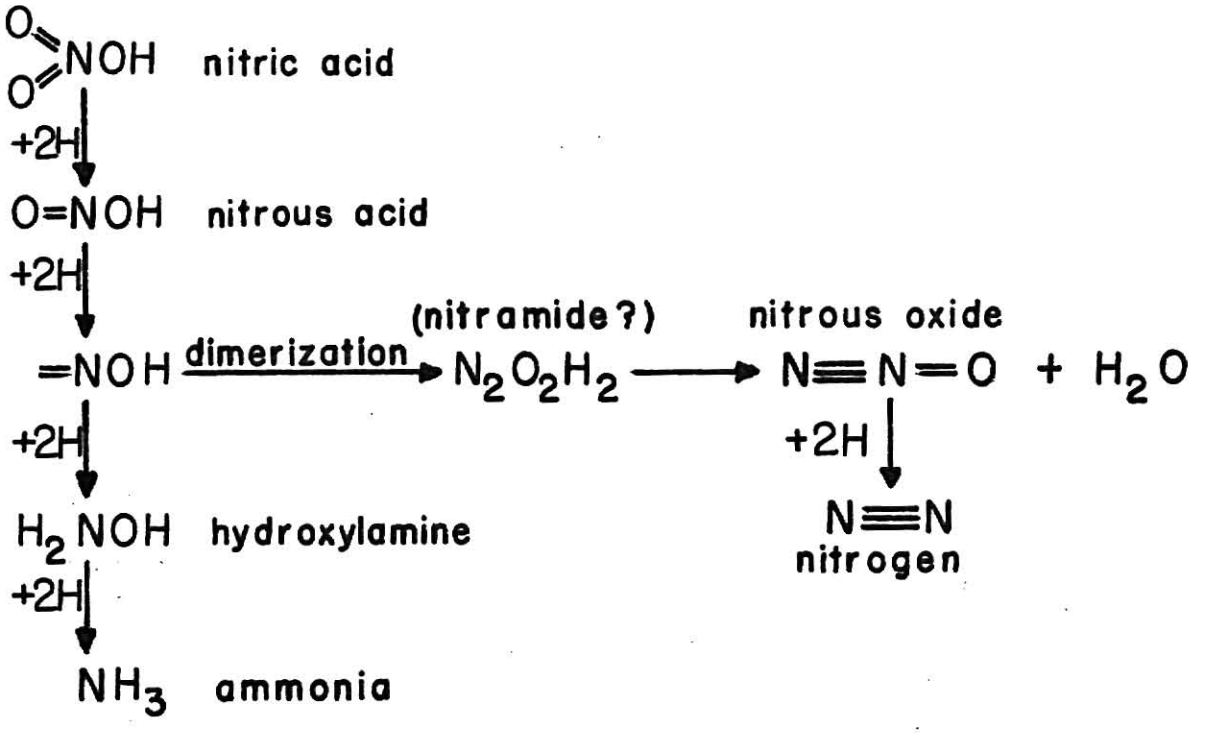


Fig. 1

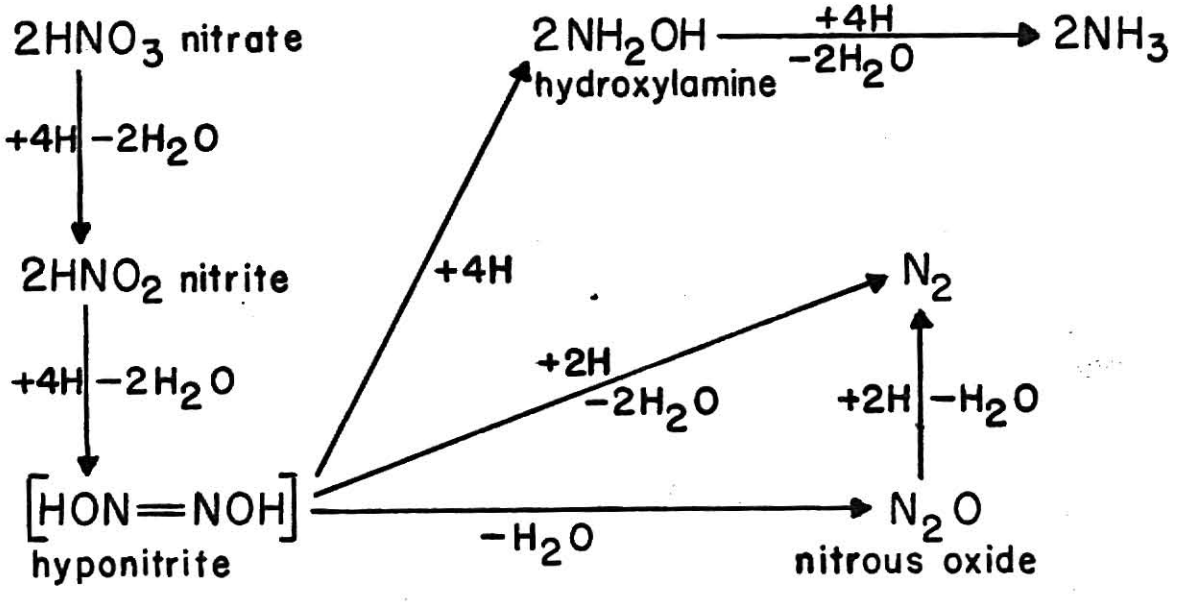


Fig. 2

EXPLANATION OF PLATE III

Fig. 1. Soil column with soil and manure in place.

Fig. 2. Soil column illustrating sampler positions.

PLATE III

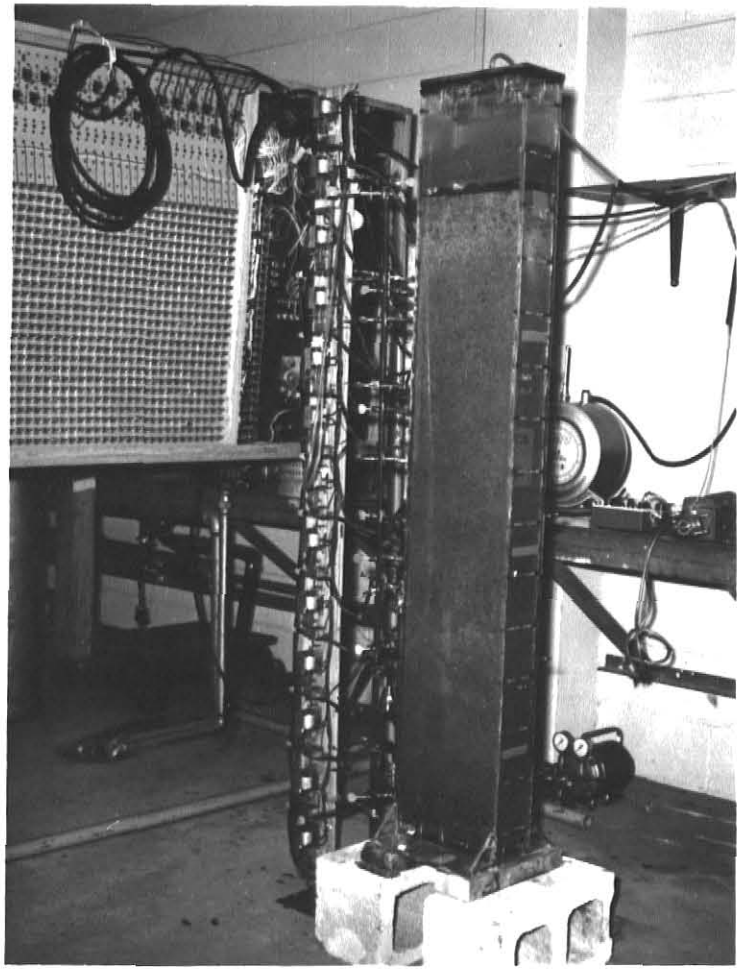


Fig. 1

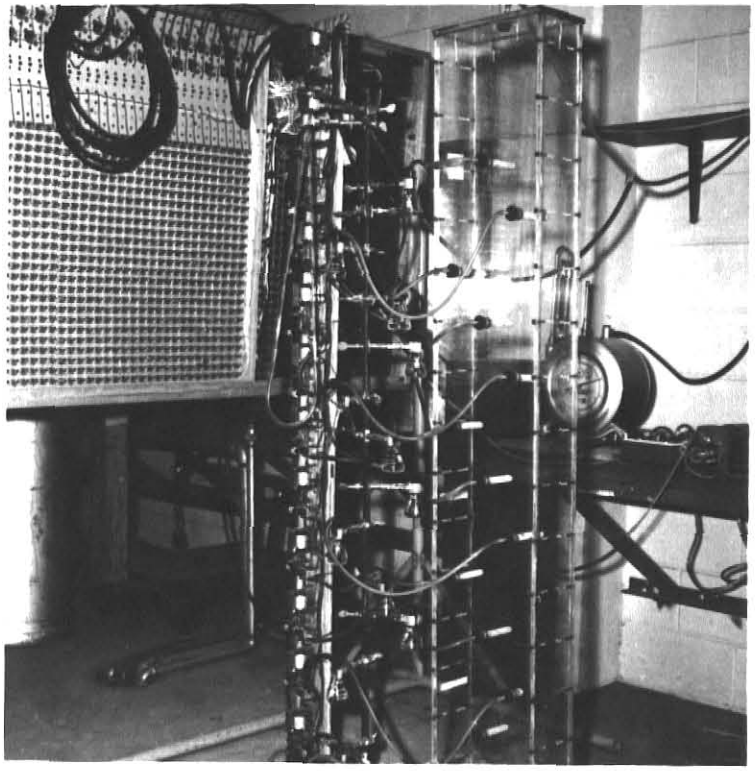


Fig. 2

EXPLANATION OF PLATE IV

Fig. 1. Entering gas meter, tubing pump, and gas chromatograph with strip chart recorder.

Fig. 2. Exiting wet test gas meter with gas sampling controls.

PLATE IV

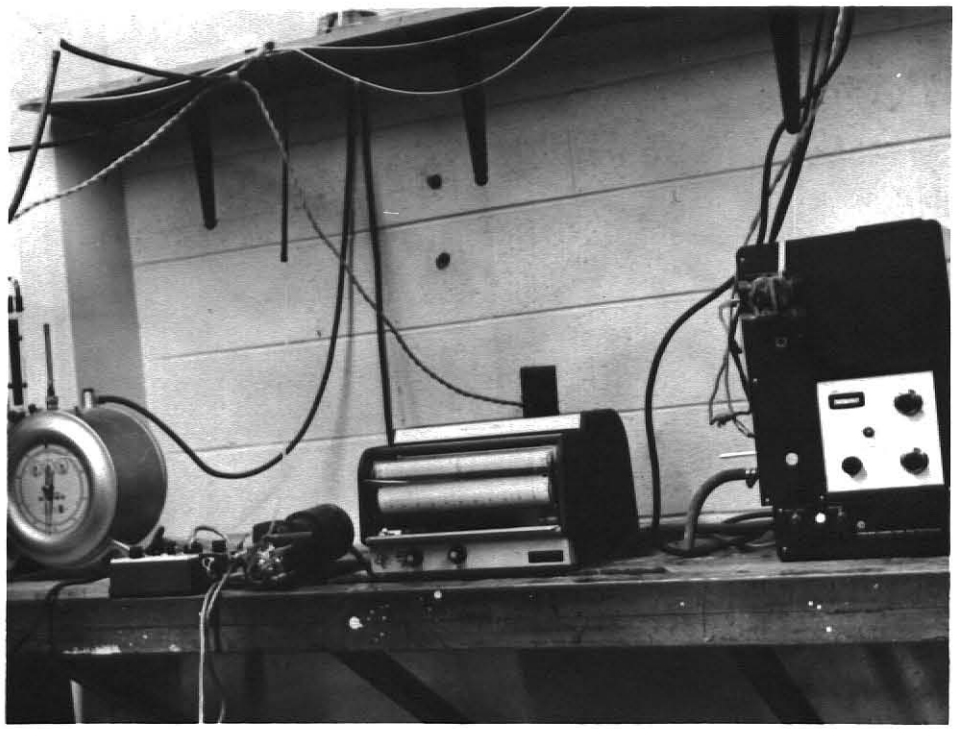


Fig. 1

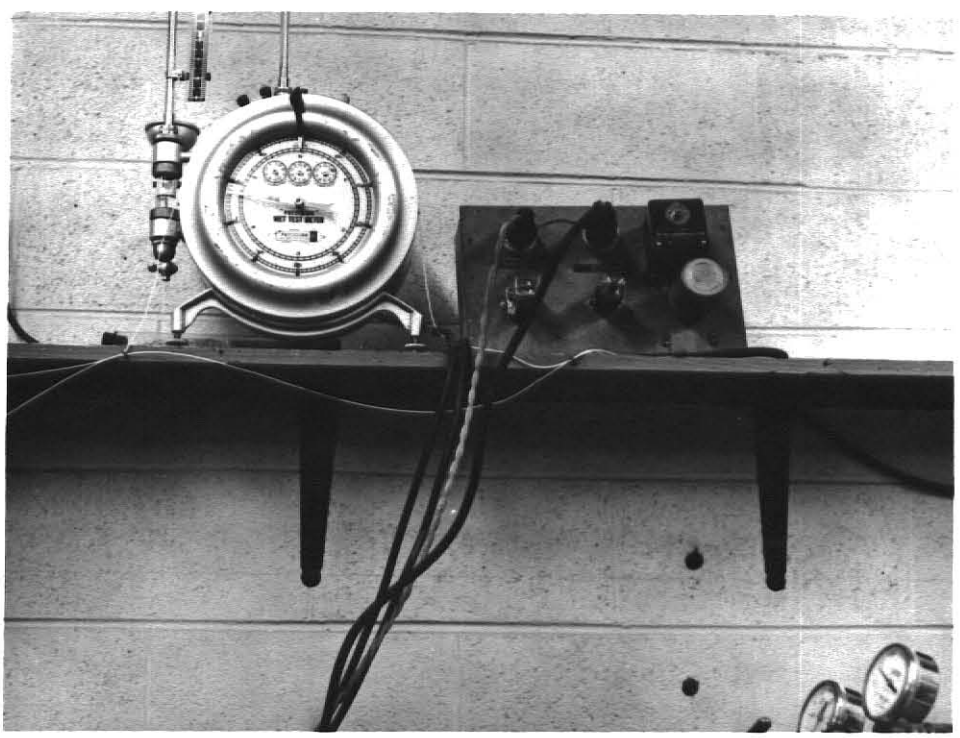
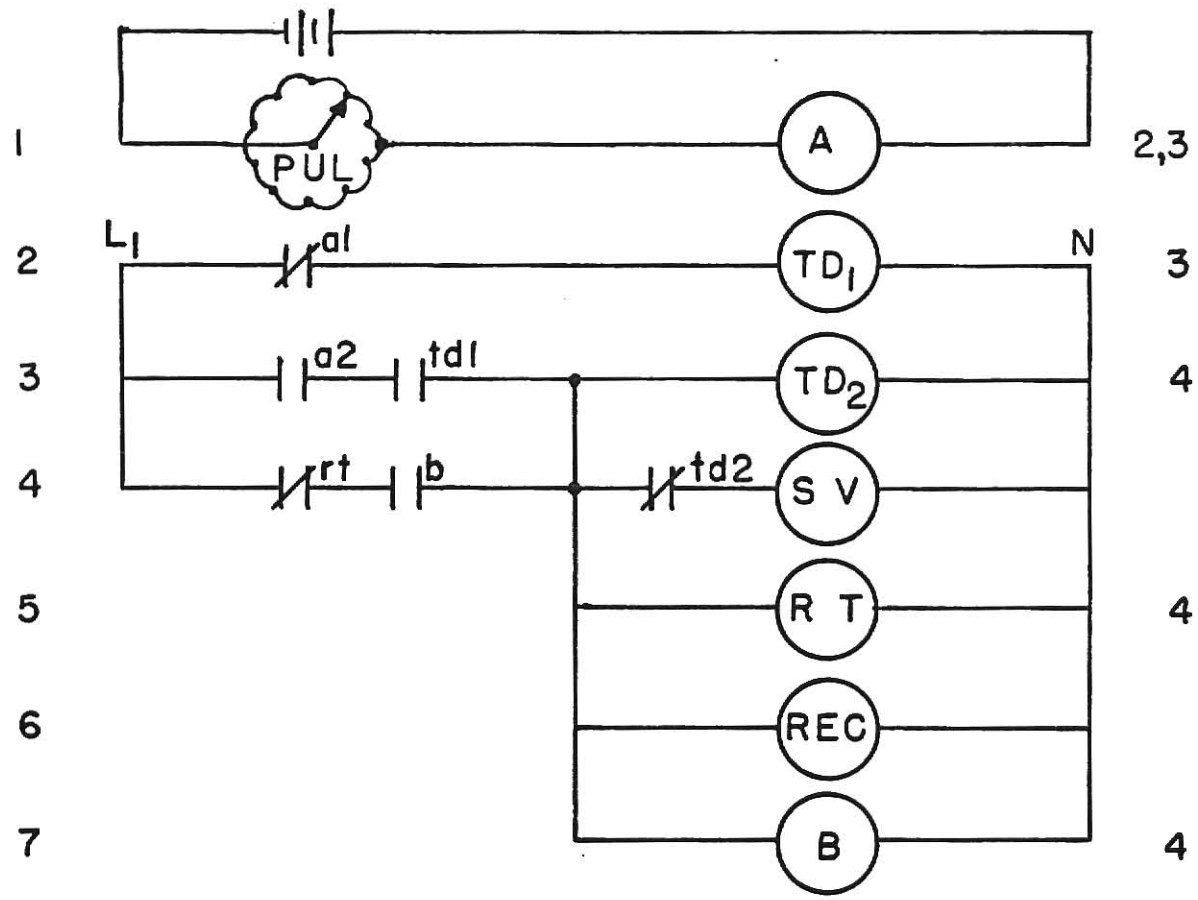


Fig. 2

EXPLANATION OF PLATE V

Gas sampling and analysis control circuit.



A - SPDT Relay

B - SPST Relay

*TD_1 - N.O. Thermal delay relay. Function is to provide delayed opening of contacts (td_1) after TD_1 is de-energized.

*TD_2 - N.C. Thermal delay. Function is to provide delayed opening of contacts (td_2) after TD_2 is energized.

SV - Solenoid Value

RT - Reset Timer - opens when timed out. Resets for new timing interval almost immediately.

REC - Recorder

PUL - Pulsing contacts on gas meter

* - TD_1 and TD_2 being thermal delays have a timing period after being energized and another after being de-energized before contacts are activated or deactivated.

RESULTS

The results of the 13 test runs show few solid facts about denitrification. They do indicate that part of the apparatus has great potential and that other parts need further development and experimentation. The gas measuring and analysis part of the unit definitely did not produce useable data. The water sampling system, on the other hand, produced what appears to be quite good data. As mentioned earlier, the instruments which were to have measured the moisture level of the soil were not usable and therefore no true correlation can be attempted between this parameter and nitrogen transformations in the soil.

The main problem with the gas system probably lies in the volume measurement. The normal flow rates produced by the sampling vacuum pump are much below the recommended minimum rate for the wet test volume meters. Experiments comparing the measurements by these volume meters with an air displacement standard under steady flow rates indicated that the meters gave good results. Their results apparently break down under intermittent flow such as existed during sampling conditions. Another problem was the existence of very small but very troublesome leaks around the top plate in the soil column. These two problems combined to completely invalidate all of the gas data and eliminate any hope of running a total nitrogen balance on the soil column.

The water sample data and the soil and manure analysis data taken before and after the test runs show some interesting facts. Less than 10% of the total nitrogen lost from the soil was leached

from the soil in the water samples. This would indicate that drawing off of water samples at various positions down the column removed very little of the soil nitrogen from the scene of active transformations. Only about 2% of the nitrogen lost from the column was leached out of the bottom of the 4-foot soil profile and under no conditions was the nitrate nitrogen concentration above 5 mg/l in the water drawn out of the bottom of the column.

The soil and manure analysis data indicate that 5.60 grams of nitrogen was lost from the soil during the 13 test runs. Only 0.48 grams of this loss occured due to leaching. This leaves an unaccounted for loss of nitrogen of 5.12 grams. The soil pH was above 8 and the soil moisture level was probably relatively high so there is little reason to doubt that the loss of soil nitrogen was due to microbial denitrification. Another interesting fact is that although 5.60 grams of nitrogen were lost from the soil column, only 2.08 grams of nitrogen were lost from the manure itself. Addition of the manure to the soil as well as the other environmental conditions existing in the soil apparently stimulated the mineralization of organic nitrogen already present in the soil and the subsequent denitrification of the resulting nitrogen compounds. A partial explanation is that larger microbial numbers developed due to the presence of a concentrated energy supply in the form of manure More nitrogen was actually lost from the total soil profile than was added in the manure layer.

The water sample data indicate that the nitrate concentrations in the soil were relatively high before the runs were started. Concentrations dropped to a low level during the first few runs

Table I

NITROGEN BALANCE SHEET

Nitrogen at start:	
in soil	17.23 gr.
in manure	4.46 gr.
total	21.69 gr.
Nitrogen removed from soil:	
in water	.48 gr.
in air	?
Nitrogen at finish:	
in soil	13.71 gr.
in manure	2.38 gr.
total	16.09 gr.

Total N lost from soil = 21.69 - 16.09 = 5.60 gr.

Unexplained loss of N = 5.60 - .48 = 5.12 gr.

N lost from manure = 4.46 - 2.38 = 2.08 gr.

and stayed low until about run 9. Relatively anaerobic conditions existed in the soil because of the high soil moisture level and the small amount of air entering the soil during runs 2, 3, 4, 5 and 6. This anaerobic condition was verified by sampling the soil gases at each water sampling position and analyzing them with the gas chromatograph. The oxygen level was very low at all sampling positions while CO_2 and methane levels were high. The nitrate levels in the water samples began to rise when the control panel was set to draw more air into and through the column from run 7 on through the remainder of the test runs. The oxygen levels in the soil were verified by sampling again and were high at the upper levels and decreased with depth. Nitrite levels are closely related to nitrate levels and were high only after the nitrate levels became high.

Ammonia levels as indicated by the water samples were low all through the column when tests were started except right below the manure layer. The point of highest ammonia concentrations remained at the manure layer until after run 7 when the points of highest concentration started shifting towards lower levels in the column. This evidently was related to the change in aeration of the soil. The introduction of more oxygen allowed the aerobic ammonifiers to mineralize the nitrogen of residual organic matter in the soil as well as any organic nutrients leached from the manure layer. The movement of the region of highest ammonia concentrations to greater depths in the soil column during the last few runs preceded narrowly a similar movement by the region of highest nitrate and nitrite concentrations. The final soil analysis

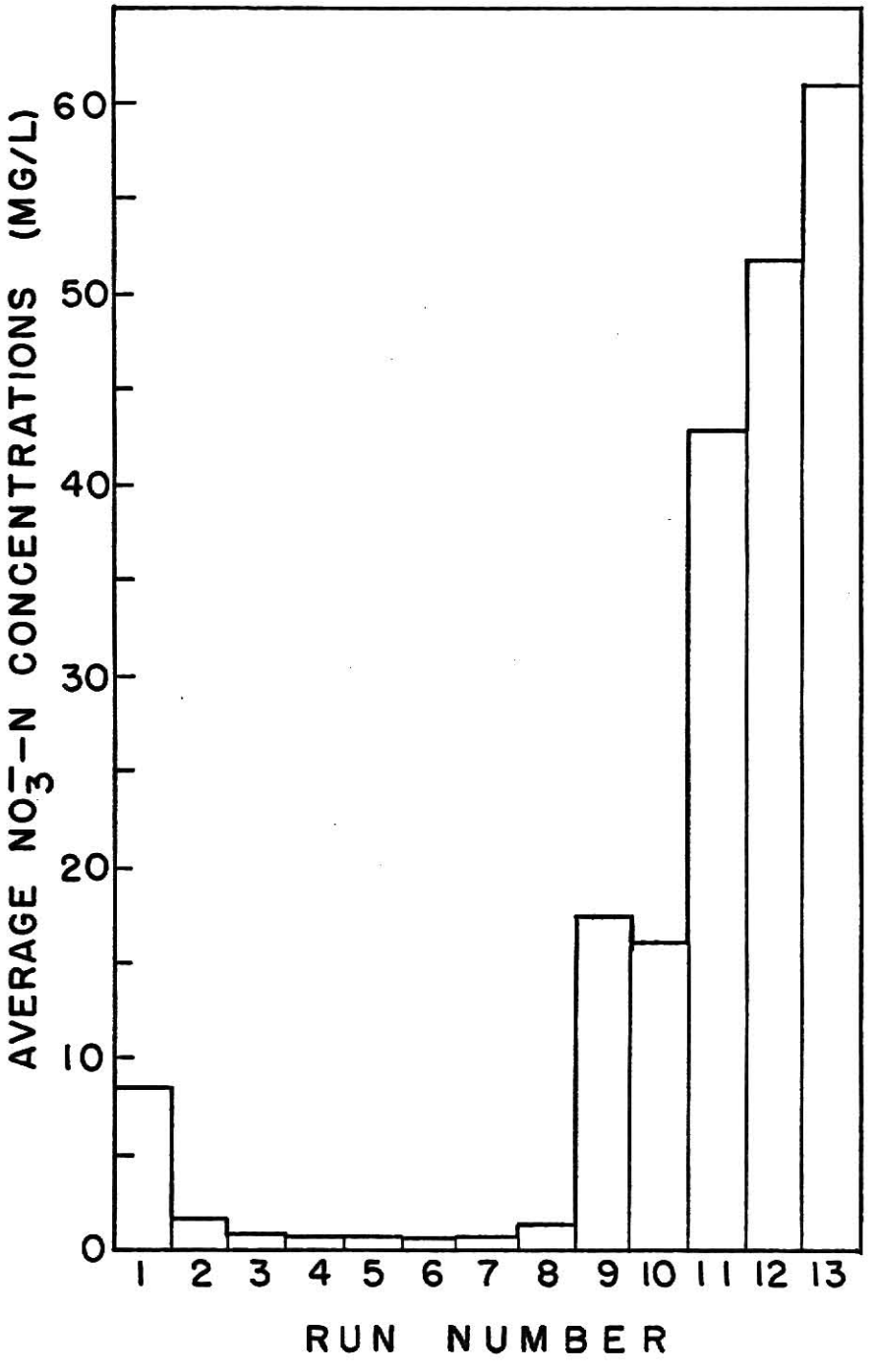


Fig. 1 AVERAGE NITRATE CONCENTRATIONS IN WATER SAMPLES

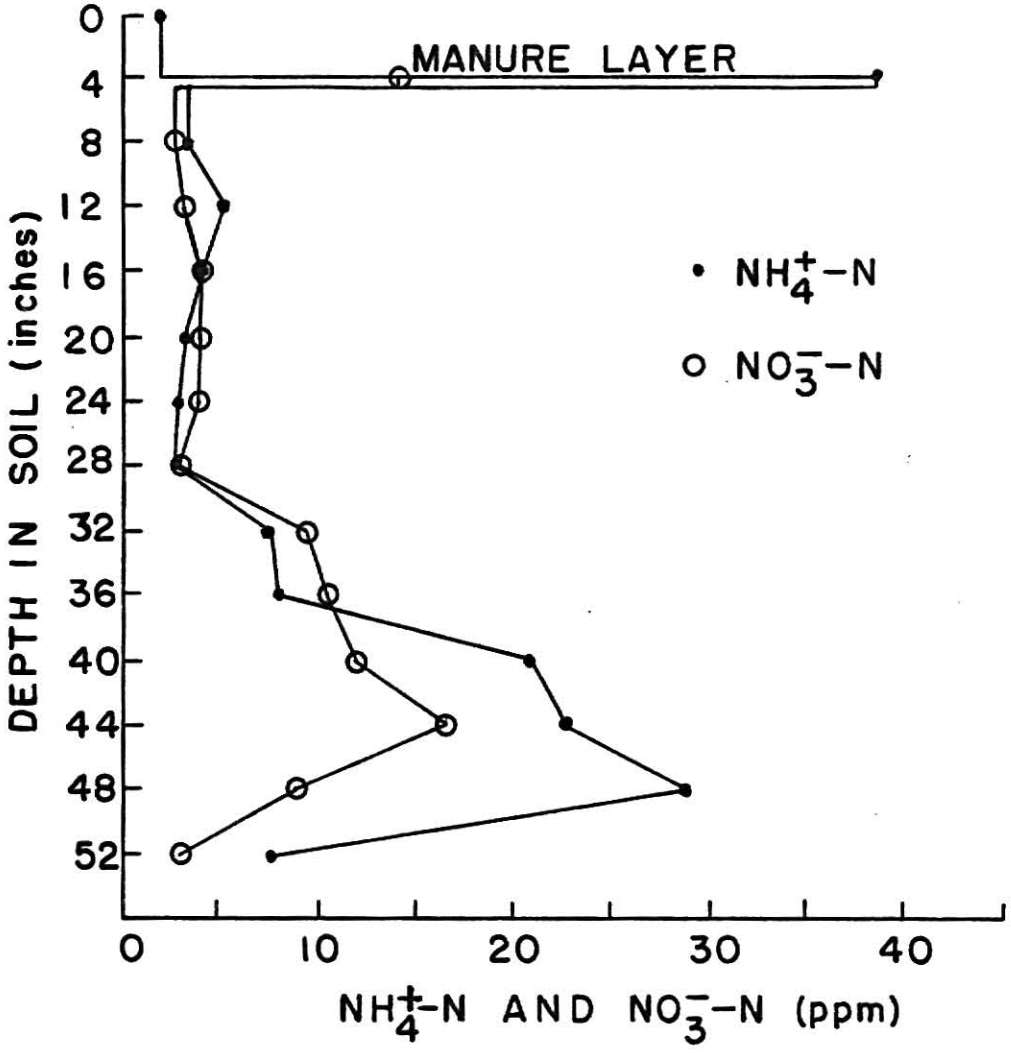


Fig. 2 NITRATE AND AMMONIUM LEVELS IN THE SOIL AT THE END OF TESTING

indicates that the organic matter levels are closely related to the location of the region of active ammonification. The organic matter levels are low above this region and are high within and below this region.

CONCLUSIONS

It appears that our apparatus can furnish some valuable information about nitrogen transformations but it still needs some improvements. Control and monitoring of the soil moisture level and gas volumes are important to the discovery of useful relationships concerning denitrification as a nitrate pollution control. Collection of water samples is very flexible with the use of the sequence control panel. These samples can be taken with a minimum of disturbance to the soil reactions and they give very informative data with respect to the sight of mineralization and nitrification. The sight of these two microbial reactions is definitely controlled by the aeration status of the soil. Extensive denitrification apparently occured in the soil column and the presence of manure in the soil column stimulated microbial activity to such an extent under the conditions existing in the column that more nitrogen was actually lost due to denitrification than was added in the manure. This apparatus should closely approximate the ideal if further work can develop equipment and procedures to monitor and control the soil moisture level and aeration of the soil in the soil profile. All other variables including soil temperature can be controlled well.

SUGGESTIONS FOR FURTHER STUDY

This apparatus and field of interest obviously contain considerable potential and thus merit further study. The apparatus will come close to combining the best of the 3 afore mentioned methods of studying denitrification if the soil moisture level and soil aeration status are closely controlled and monitored. One possible method of monitoring the soil moisture level is the use of tensiometers inserted into the soil profile at 3 or 4 levels. A method of measuring gas volumes under intermittent flows is needed. Perhaps some method of measurement by water displacement where pressure differentials are minimal can be devised. Gas quantification could be improved by several steps. One would be the removal of the gas scrubber bottles from the lines as they furnish no useful information which the chromatograph is unable to furnish and thus act only as gas mixing chambers. Another improvement would entail the thorough flushing of all liquid sample bottles and gas lines with the helium carrier gas of the gas chromatograph. Another indicator of the soil aeration status could be gained if necessary by periodic sampling of the soil atmosphere at each water sampling position by use of a gas sampling syringe. The surface soil conditions could be made to more nearly approach natural conditions if sufficient air were to flow through the top of the soil column so as to maintain the relative humidity and oxygen content of the air above the soil at more normal values. This could easily be done with an air pump and a volume meter to measure flow out the top of the column. The gases flowing in and

out of the top of the column could be periodically quantified by an automated sampling circuit to the chromatograph.

Once the soil column and its accompanying equipment are perfected, studies can be easily run in which different parameters are varied. The parameters of interest include temperature, moisture level, substrate level, soil pH, soil structure, and soil aeration. Different manure application rates should be tried. The effects of a layer of manure versus mixing the manure with the top-soil should be investigated. It needs to be determined whether denitrification can volatolize more nitrogen under the practice of periodic flooding of the soil or under a certain constant soil moisture level. The best moisture conditions for denitrification to occur will undoubtedly depend upon the soil type and texture so this relationship needs to be determined. As with all laboratory experiments, the results obtained in the lab may not be 100% applicable to field situations. This apparatus should be capable of duplicating field conditions fairly well with some of the above mentioned additions and modifications or hopefully with modifications similar in simplicity. If this is so, then the results obtained should be quite applicable to field conditions, but their applicability in the field should be checked never-the-less.

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APPENDIX

TABLE 2
Soil Sample Analysis

Sample Description	Depth in Soil Column (inches)	pH	O.M. %	Total N %	NH_4^+-N ppm	NO_5^--N ppm
Composite sample taken before testing			.15	.03	3.3	1.7
Final Samples						
1	0-4"	8.3	.2	.023	1.9	.7
2	4"	7.3	9.6	.73	38.6	14.1
3	4-8	8.1	.6	.022	3.4	2.8
4	8-12	8.1	.3	.022	5.1	3.1
5	12-16	8.1	.2	.023	4.2	4.2
6	16-20	8.1	.2	.023	3.1	3.9
7	20-24	8.3	.3	.019	2.8	3.9
8	24-28	8.6	.25	.025	2.6	2.8
9	28-32	8.3	.3	.018	7.6	9.3
10	32-36	8.2	.3	.025	7.9	10.5
11	36-40	8.4	.35	.023	21.0	11.9
12	40-44	8.3	.45	.024	22.7	16.7
13	44-48	8.4	.35	.048	28.9	8.8
14	48-52	8.5	.5	.024	7.6	2.8

TABLE 3

Nitrate Concentrations
(mg/l-N)

Run #	Sampling Position 1	2	3	4	5	6	7	8	9	10	11	12	13	Total	Average
1	2.45	.85	0.00	18.00	14.70	0.00	16.50	17.40	16.65	17.55	9.75	0.00	3.30	117.75	8.41
2	2.05	2.15	2.40	2.00	2.15	2.10	1.55	1.75	1.55	.50	.50	.40	.25	19.35	1.49
3	.55	.60	.95	1.40	1.65	.70	.70	1.65	1.00	.50	.95	.55	.45	11.65	.80
4	.75	1.00	.85	.65	.75	.65	.65	.75	.75	.80	.85	.60	.95	10.60	.76
5	.80	.80	.70	.70	.75	.70	.65	.75	.70	.70	.75	.70	.65	9.35	.72
6	.80	.70	.60	.65	.55	.70	.60	.70	.65	.65	.70	.70	.70	8.70	.67
7	.80	.75	.70	.73	.67	.67	.74	.84	.75	.74	.80	1.20	.15	9.94	.71
8	3.30	4.20	2.20	2.60	2.00	.90	.15	.05	0.00	.10	.05	.15	.25	15.95	1.23
9	4.60	31.00	40.00	65.00	70.00	18.00	.40	1.30	0.30	.45	.45	.40	.20	243.60	17.40
10	.90	17.40	78.75	25.00	57.00	13.80	12.50	10.40	.55	.25	.30	.30	.40	225.05	16.08
11	5.10	33.50	91.70	172.50	142.50	90.00	17.30	15.80	3.70	.40	.40	.50	.40	594.80	42.49
12	4.70	13.40	29.00	43.50	72.00	180.00	153.30	113.30	51.00	.40	3.10	.70	4.40	727.10	51.94
13	6.60	6.80	14.40	23.00	20.50	30.60	220.00	210.00	150.0	48.80	55.00	.50	5.00	851.20	60.80
Total	33.40	113.15	262.25	355.73	385.22	338.82	425.04	374.69	227.6	71.84	73.60	6.70	17.10	2845.04	203.60
Average	2.57	8.70	20.17	27.36	29.63	26.06	32.70	28.80	17.50	5.53	5.66	.52	1.32	218.82	

TABLE 4

Nitrate Totals
(mg-N)

Sampling Positions

Run #	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
1	.125	.048	.000	.504	.220	.000	.330	.331	.383	.825	.517	.000	5.122	9.490
2	.109	.105	.122	.110	.114	.109	.076	.094	.081	.026	.026	.018	.419	1.409
3	.027	.029	.047	.067	.081	.036	.033	.081	.053	.026	.048	.024	.610	1.162
4	.034	.050	.042	.034	.037	.032	.032	.035	.036	.042	.042	.027	.095	1.294
5	.037	.038	.035	.037	.038	.036	.031	.036	.034	.036	.037	.035	.917	1.347
6	.037	.034	.030	.032	.027	.036	.031	.034	.030	.034	.036	.036	.816	1.213
7	.036	.037	.034	.038	.034	.035	.033	.039	.039	.038	.038	.061	.272	.782
8	.152	.101	.044	.143	.012	.005	.005	.001	.000	.002	.003	.007	.345	.820
9	.212	1.457	2.000	3.380	3.290	.972	.020	.061	.016	.022	.023	.018	.250	13.078
10	.041	.887	4.016	1.350	2.850	.731	.563	.520	.029	.013	.015	.014	.484	12.421
11	.235	1.608	4.309	9.315	6.983	4.860	.865	.695	.185	.021	.020	.023	.516	31.903
12	.212	.616	1.334	2.132	3.816	9.540	7.665	5.665	2.652	.021	.143	.033	5.676	45.967
13	.323	.333	.648	1.219	1.066	1.530	11.660	10.500	7.950	2.537	2.750	.026	5.315	51.917
Total	1.580	5.343	12.661	18.361	18.568	17.922	21.344	18.092	11.488	3.643	3.698	.322	20.837	172.812

TABLE 5

Ammonia Concentrations
(mg/l-N)

Sampling Positions

Run #	1	2	3	4	5	6	7	8	9	10	11	12	13	Total	Average
1	450.00	265.00	0.00	2.40	3.40	0.00	1.30	1.30	1.35	1.40	1.60	0.00	1.52	729.97	52.14
2	36.00	95.00	89.00	24.20	31.70	15.20	5.30	7.40	4.10	1.40	2.10	1.90	1.45	314.75	24.21
3															
4															
5	77.00	35.50	34.70	30.80	44.30	25.20	5.10	5.00						257.60	32.20
6	110.00	99.00	55.00	71.00	81.50	69.50	27.00	36.00	12.00	10.00	12.00	16.00		599.00	49.92
7	81.00	75.00	53.00	66.00	76.00	61.00	23.00	37.00	13.50	8.50	9.00	7.50	10.60	552.35	39.45
8	28.00	41.00	34.00	52.00	41.00	26.00	22.00	26.00	17.00	6.00	4.00	3.75	4.40	305.15	23.47
9	23.00	27.00	39.00	73.00	85.00	60.00	32.00	46.00	22.00	4.50	4.50	2.50	4.00	462.00	33.00
10	28.50	10.00	29.00	74.00	97.00	85.00	59.00	81.00	40.00	8.00	11.00	3.00	6.20	560.70	40.05
11	2.20	1.60	1.00	48.00	55.00	88.00	68.00	85.00	44.00	14.00	18.50	7.30	7.00	527.60	37.69
12	0.80	.60	.80	.60	.60	19.00	90.00	118.00	76.00	24.00	31.00	6.60	10.00	399.00	28.50
13	1.40	.80	.80	1.00	.80	1.00	42.00	36.00	82.00	41.00	62.00	8.60	11.40	311.80	22.27
Total	837.90	388.15	336.30	443.00	516.30	451.20	420.60	478.70	311.95	118.80	155.70	57.15	119.5		
Average	76.17	35.29	30.57	40.27	46.94	41.02	38.23	43.52	31.19	11.88	15.57	5.72	13.29		

TABLE 6

Ammonia Totals
(mg-N)

Sampling Positions

Run #	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
1	22.950	14.810	0.000	.067	.051	0.000	.026	.025	.031	.066	.085	0.000	2.359	41.765
2	1.908	4.655	4.539	1.331	1.680	.790	.260	.400	.213	.073	.107	.087	2.429	18.472
3														
4														
5	.350	.170	.170	.160	.230	.130	.020	.020						1.250
6	.510	.480	.280	.390	.400	.350	.140	.180	.060	.050	.060	.080		2.980
7	3.650	3.680	2.600	3.430	3.800	3.170	1.040	.170	.700	.430	.420	.380	19.186	47.966
8	1.290	.980	.680	2.860	.250	.160	.700	.750	.390	.090	.200	.170	6.070	14.590
9	1.058	1.269	1.950	3.796	3.995	3.240	1.600	2.162	1.166	.221	.225	.113	5.000	30.456
10	1.311	.510	1.479	3.996	4.850	4.505	2.655	4.050	2.080	.424	.561	.138	7.502	37.570
11	.101	.077	.047	2.592	2.675	4.752	3.400	3.740	2.200	.728	.907	.336	9.030	33.305
12	.036	.028	.037	.029	.032	1.007	4.500	5.900	3.952	1.248	1.426	.310	12.900	33.736
13	.069	.039	.036	.053	.042	.050	2.226	1.800	4.346	2.132	3.100	.447	12.118	28.781
Total	33.233	26.728	11.818	18.704	18.025	18.154	16.567	20.727	15.138	5.462	6.433	2.061	76.594	

TABLE 7

Nitrite Concentrations
(mg/1-N)

Sampling Positions

Run #	1	2	3	4	5	6	7	8	9	10	11	12	13	Total	Average
1															
2															
3															
4															
5															
6															
7															
8															
9	.25	4.25	2.50	7.50	5.98	1.25								27.730	3.96
10		5.50	3.00	2.25	4.75	.50	.05	.45						34.000	4.25
11		.25		2.375	.75	.875	.125	.125						16.375	2.34
12				.125	.20	7.50	1.50	1.75	.50					13.825	1.975
13				.025			37.50	50.00	30.00	2.25	6.25			139.525	19.93
Total	.25	10.00	5.50	12.275	11.65	10.125	39.175	52.325	30.50	2.25	6.25				
Average	.25	3.33	2.75	2.46	2.92	2.53	9.79	13.08	15.25	2.25	6.25				

TABLE 8

Nitrite Totals
(mg-N)

Sampling Positions

Run #	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
1														
2														
3														
4														
5														
6														
7														
8														
9	.012	.200	.125	.390	.281	.068								1.784
10		.281	.153	.121	.238	.026	.002	.022						2.963
11		.012		.128	.037	.047	.006	.006						1.519
12				.006	.011	.398	.075	.088	.026					.854
13				.001			1.950	2.500	1.590	.117	.313			7.835
Total	.012	.493	.278	.646	.567	.539	2.033	2.616	1.616	.117	.313			14.955

TABLE 9

Totals of Nitrogen Concentrations
(mg/l-N)

Sampling Positions

Run #	1	2	3	4	5	6	7	8	9	10	11	12	13	Total	Average
1	452.45	265.85	0.00	20.40	18.10	0.00	17.80	18.70	18.00	18.95	11.35	0.00	4.82	847.72	60.55
2	38.05	97.15	91.40	26.20	33.85	17.30	6.85	9.15	5.85	1.90	2.60	2.30	1.70	334.30	25.72
3	.55	.60	.95	1.40	1.65	.70	.70	1.65	1.00	.50	.95	.55	.45	11.65	.89
4	.75	1.00	.85	.65	.75	.65	.65	.75	.75	.80	.85	.60	.95	10.60	.75
5	77.80	36.30	35.40	31.50	45.05	25.90	5.75	5.75	.70	.70	.75	.70	.65	266.94	20.53
6	110.80	99.70	55.60	71.65	82.05	70.20	27.60	36.70	12.65	10.65	12.70	16.70	.70	607.70	46.75
7	81.80	75.75	53.70	66.73	76.67	61.67	23.44	37.84	14.25	9.24	9.80	8.70	10.75	561.99	40.14
8	31.30	45.20	36.20	54.60	43.00	26.90	22.15	26.85	17.00	6.10	4.05	3.90	4.65	321.10	24.70
9	27.85	62.25	81.50	145.50	160.98	79.25	32.40	47.30	22.30	4.95	4.95	2.90	4.20	733.33	52.38
10	29.40	32.90	110.75	101.25	158.75	99.30	71.55	91.95	40.55	8.25	11.30	3.30	6.60	819.85	58.56
11	7.30	35.35	92.70	222.88	198.25	178.87	85.42	100.925	47.70	14.40	18.90	7.80	7.40	1075.78	76.84
12	5.50	14.00	37.00	44.325	72.80	206.50	244.80	233.05	127.50	24.40	34.10	7.30	14.40	1147.13	81.94
13	8.00	7.60	15.20	24.025	21.30	31.60	299.50	296.00	262.00	92.05	123.25	9.10	16.40	1302.53	93.04
Total	871.55	773.65	611.25	811.01	913.20	798.84	838.62	905.82	570.25	192.89	235.55	63.85	73.67	8040.63	574.33
Average	67.04	59.51	47.02	62.39	70.25	61.45	64.51	69.68	43.87	14.84	18.12	4.91	5.67	618.51	

TABLE 10

Total Nitrogen
(mg-N)

Sampling Positions

Run #	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
1	23.075	14.887	0.00	.571	.272	0.000	.356	.356	.414	.891	.602	0.000	7.481	51.255
2	2.017	4.760	4.661	1.441	1.794	.899	.336	.494	.294	.099	.133	.105	2.848	19.881
3	.027	.029	.047	.067	.081	.036	.033	.081	.053	.026	.048	.024	.610	1.162
4	.034	.050	.042	.034	.037	.032	.032	.034	.036	.042	.042	.027	.095	1.295
5	.387	.208	.205	.197	.268	.166	.051	.056	.034	.036	.037	.035	.917	2.597
6	.547	.514	.310	.422	.427	.386	.171	.214	.090	.084	.096	.116	.816	4.193
7	3.686	3.717	2.634	3.468	3.834	3.205	1.073	1.739	.739	.468	.458	.441	19.457	48.747
8	1.442	1.081	.724	3.003	.262	.165	.705	.751	.390	.092	.203	.177	6.415	15.510
9	1.282	2.926	4.075	7.566	7.566	4.280	1.620	2.223	1.182	.243	.248	.131	5.250	43.518
10	1.352	1.678	5.648	5.467	7.938	5.262	3.220	4.592	2.109	.437	.576	.152	7.986	52.954
11	.336	1.697	4.356	12.035	9.715	9.659	4.271	4.441	2.385	.749	.927	.359	9.546	66.727
12	.248	.644	1.371	2.167	3.859	10.945	12.240	11.653	6.630	1.269	1.569	.343	18.576	80.566
13	.392	.372	.685	1.273	1.108	1.580	15.836	14.800	13.886	4.786	6.163	.473	17.433	88.533
Total	24.830	32.190	24.76	37.710	37.160	36.620	39.940	41.400	28.240	9.220	11.100	2.380	97.430	

DEVELOPMENT OF A NITROGEN BALANCE IN A LABORATORY SOIL PROFILE WITH A HEAVY APPLICATION OF BEEF CATTLE WASTES

by

JOHN ALLEN GEORGE

B. S., Kansas State University, 1969

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ABSTRACT

One of the major problems associated with the disposal of large amounts of animal and other wastes on small land areas is the possible pollution of surface and ground waters with inorganic nitrogen compounds. The nitrate ion, which may evolve from the mineralization of nitrogen found in organic matter, is very mobile in the soil solution and is a potentionally dangerous water pollutant.

It has long been known that nitrogen in the form of nitrate may be lost from the soil due to denitrification. Recent work with the disposal of large amounts of wastes on small land areas has raised interest in denitrification as a method of preventing nitrate pollution of our water supplies. The purpose of this research project was to develop equipment and methods for determining a nitrogen balance in a soil profile with a heavy application of beef cattle wastes.

A soil column was constructed such that water was percolated through the soil. Water and gas samples were drawn off down the side of the column. The water samples were drawn on a time schedule controlled by a sequence control panel. Water was added to the soil surface once a week for 13 weeks, and water and gas samples were taken. A gas chromatograph was used to analyze the soil gases. The nitrogen forms in the water samples were quantified chemically. The quantities of nitrogen in the soil before testing and after testing were to be combined with the results of the water sample analysis and the gas analysis to determine a total nitrogen balance for the soil profile.

The results indicate that parts of the equipment worked quite well, while other parts need further development. The gas measuring system gave little or no useful data, but the soil and water analysis gave excellent data. The water sampling control panel was flexible enough to allow good water sampling without undue disturbance of the soil processes. The soil and manure analysis data indicated that a considerable loss of nitrogen from the soil column occured, but the gas analysis system was not capable of collaboration. Less than 10% of the total nitrogen lost from the soil was leached out in the water samples, indicating that the drawing off of water samples removes a minimum of nitrogen from the sight of active transformations. Only about 2% of the total indicated nitrogen loss was leached out of the bottom of the 4-foot soil profile. The nitrate concentration was never above 5 mg/l in this water.

The equipment and procedures used indicate considerable potentia for studying nitrogen transformations in the soil. A workable gas analysis and quantification system needs to be developed before the actual soil conditions can be accurately controlled and a useful relationship between the various parameters can be determined. The value of further results obtained with this apparatus will increase as the detail with which field conditions are duplicated is improved.