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Effect of Controlled Redox Potential and pH on Heterotrophic Nitrogen Fixation and Mineralization in Crowley Silt Loam Soil.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Laboratory investigations were carried out to study the magnitudes and rates of heterotrophic N_2 fixation and mineralization of organic N as influenced by controlled redox potentials and pH values. Preincubated Crowley silt loam soil suspensions was amended with 0.50% labelled rice straw (41% ^{15}N excess) and incubated for six weeks in the dark. Effects of all combinations of four redox potentials (-100, +100, +300, and +500 mV) and two pH values (5 and 7) were studied. At weekly intervals, soil suspension samples were withdrawn and analysed for N_2 fixation by using the C_2H_2 reduction assay, total organic N, extractable NH_4^+-N, NO_3^--N, labelled organic N, and labelled extractable NH_4^+-N.

The greatest production of C_2H_4 was recorded in the -100 mV treatment at all weekly samplings, and the smallest C_2H_4 production rate was found at +500 mV. The magnitude of C_2H_4 production was about twice as great under anaerobic conditions (6.281 μmoles C_2H_4/kg/week) as under aerobic conditions (3.630 μmoles C_2H_4/kg/week). Compared to production of C_2H_4 at pH 7, that at pH 5 showed a net decrease (7.9 μmoles of C_2H_4/kg/week (-100 mV and pH 7) and 5.6 μmoles C_2H_4/kg/week (-100 mV and pH 5). At the end of the experiment, the production of C_2H_4 at -100 mV was significantly greater than those at all other redox potentials. The average C_2H_4 production rate corresponded to a N_2 fixation rate of 10 kg/ha/year if a 3:1 ratio is assumed. The amount of extractable NH_4^+-N released at -100 mV over time was significantly greater than the NH_4^+-N released at the other redox potential and pH levels. Significantly greater amounts of NH_4^+-N were released at pH 5 compared to pH 7. The NH_4^+-N released at the end of the incubation period was 55 mg N/kg soil at -100mV and pH 7 compared to 70 mg N/kg soil at the same redox level and pH 5. The nitrification rate was slightly greater at +500 mV than +300 mV and was significantly greater at pH 7 than pH 5, resulting in a significant redox potential by pH interaction. No nitrification was recorded at the +100 and -100 mV treatments. The amount of labelled organic N mineralized at pH 5 was about twice that mineralized at pH 7 (0.79 mg ^{15}N/kg soil). A significant redox potential by pH

interaction affected the amounts of labelled NH_4^+-N released. The labelled N unaccounted for ranged between 2 to 24 % of the total ^{15}N added to the soil.

INTRODUCTION

The economy of farmers of developing countries has been severely affected by the price increase in industrial N fertilizer. A great deal of research has been carried out to find alternative sources of this key nutrient element for rice. Biological N_2 fixation is one of the obvious major sources. In Asia and other parts of the tropics, rice has been grown for centuries without N fertilizer, yet yields were stable. Contrary to the N balances for dryland crops, the N balances for rice show an excess, specially where no N fertilizer was applied. This oversupply is attributed to the biological N_2 fixation and is estimated to vary from 15 to 50 kg/ha (Koyoma and App 1979). Moreover, wetland rice yields are usually greater than dryland grain crops. Patrick (1982) attributed this yield difference partly to a better water management and partly to greater N_2 fixation in the submerged soil. Wetland soils are a unique site for biological N_2 fixation. Blue greens, *Azollae* and heterotrophic bacteria are the principal agents recognized for non-symbiotic N_2 fixation in rice. Blue greens thrive in the water column and on the surface of submerged soils. *Anabena azollae* in symbiosis with the freshwater fern *Azolla* fixes N. The aerobic and anaerobic zones of wetland soils are both colonized by free-living bacteria. Blue greens are generally recognized as the most efficient N-fixers in wetland rice (Watanabe and Yamamoto, 1971; Watanabe et al., 1979). Because of the conflict between two important factors (light and anaerobiosis) for the growth and activity, Watanabe (1978) considered the actual contribution of photosynthetic bacteria doubtful. For the heterotrophic N_2 fixers, organic material is the energy source. O'Toole and Knowles (1973) reported 10-14 mg N_2 fixation for each gram of carbohydrate consumed. In rice fields, living rice roots, and crop residues serve as energy sources. For years, the role of the heterotrophic N_2-fixing bacteria have been overlooked. However, the use of increasing amounts of N fertilizer and pesticides is detrimental to the algal growth. The heterotrophic bacteria must be the

promising agents of N_2 fixation under these conditions. It is generally accepted that the N_2 fixation rate is greater under anaerobic conditions than under aerobic conditions.

To maintain and improve the soil fertility rice, residues are incorporated into the field in many regions of the world. These residues stimulate N_2 fixation and affect the N immobilization-mineralization turnover rates. The immobilization-mineralization reactions are essentially biological. The decomposition of organic matter which leads to the release of NH_4^+-N proceeds at a lower rate in an anaerobic soil than in aerobic soil (Tenny and Waksman, 1930) but the release of inorganic N from decomposing rice straw proceeds at greater rates under anaerobic than aerobic conditions (Acharya, 1935c; Sircar et al., 1940). The low requirement of anaerobic microorganisms for N makes them more efficient to attack the organically bound N early in the process of decomposition. Because of the absence of O2 under anaerobic conditions, mineralization usually stops at the NH_4^+-N stage.

Sircar et al. (1940) reported that inorganic N release from decomposing straw occurred at a greater C/N ratio under anaerobic conditions. Tusneem and Patrick (1971) and Reddy and Patrick (1984) presented similar evidence. Most of these studies were comparisons of mineralization of N under aerobic and anaerobic conditions. Reddy and Patrick (1979) investigated the effects of redox potential and pH on the distribution of added labelled $(NH_4)_2SO_4$. Yoneyama et al. (1977) studied the influence of redox potential and pH on N_2 fixation by heterotrophic bacteria in some Philippines soils. However, information about the decomposition of rice straw and non-symbiotic N_2 fixation as influenced by redox potential and pH is scarce.

The applicability of C_2H_2 reduction technique for measuring N_2 fixation, and isotope-ratio analysis for tracing N transformations make it possible to investigate the factors regulating theses processes. In the present investigation both redox potential and pH were controlled throughout the experiment which was aimed at determining in a

Crowley silt loam soil (1) the rate and extent of N_2 fixation, and (2) the rate and extent of mineralization of N using labelled rice straw.

REVIEW OF LITERATURE

1. Chemical and Biological Redox Systems in Flooded Soils

1.1 Aerobic-Anaerobic Interface

In a well drained soil, gas diffusion is sufficiently rapid to prevent O_2 deficiency or toxicity of CO_2. When a soil is submerged, water replaces air in the pore spaces, and thus O_2 diffusion is restricted. According to the figures given by Lemon and Kristensen (1960), Greenwood (1961), and Grabble (1966) O_2 diffusion is approximately four orders of magnitude slower in water than in air. Evans and Scott (1955) reported that the concentration of O_2 in water used for saturating a soil decreases to one-hundreth of its initial value in 75 minutes. Turner and Patrick (1968) witnessed the total disappearance of O_2 in four soil suspensions within 36 hrs of withdrawal of the O_2 supply. Yamane (1958) noted the absence of O_2 in two flooded rice fields at five sampling times during 3 months. In his respiration studies, Greenwood (1961) could not detect O_2 at the center of saturated soil crumbs. The low redox potential for saturated and submerged rice soils reported by Ponnamperuma (1965) and Patrick and DeLaune (1972) are further proof of the absence of O_2 in waterlogged soils and sediments. However, coarse sediments in shallow water which are low in organic matter may be well supplied with O_2 (Zobell, 1946). Pearsall and Mortimer (1939) were the first to describe the differentiation of submerged soil (a lake mud) into two distinct zones as result of limited O_2 penetration. Mortimer (1941, 1942) first thoroughly investigated the soil profile using an assembly of platinum electrodes. The thickness of the oxidized surface layer is determined by the supply of O_2 at the soil surface and the consumption rate of O_2 in the soil.

The two layers of a submerged soil can be characterized by the differences in redox potential. The various inorganic and organic redox systems in the soil contribute to this potential (Ponnamperuma, 1965). Patrick and DeLaune (1972) developed a technique for measuring the redox potential vertically through the soil profile and determined the

distribution of the reducible forms of soil components. They found that the apparent thickness of the oxidized layer differs according to the various components of the profile, with sulfur indicating the thickest oxidized zone, Mn indicating the thinnest oxidized zone, and Fe showing intermediate thickness. As duration of flooding increased and organic carbon was consumed, O_2 penetrated to a greater depth, that increasing the thickness of the oxidized surface layer. In a flooded paddy soil, lake sediment or estuarine sediment, aerobic, anaerobic and obligate anaerobic metabolism occurs simultaneously in different soil zones.

1.2 Redox Potential

Redox potential is a measure of the electrochemical potential or electron availability in chemical and biological systems (Gambrell and Patrick, 1978). Chemical species that lose electrons are oxidized and reduction occurs as a chemical species gains electrons. Thus a measure of redox potential (electron availability) indicates the intensity of oxidation or reduction of a chemical or biological system.

The reduction of the inorganic redox systems in the soil following flooding can be described in both intensity and capacity terms. The intensity factor determines the relative ease of the reduction, whereas the capacity factor denotes the amount of the redox system undergoing reduction (Patrick, 1980). The intensity factor can be represented by the free energy of the reduction, or more commonly by the equivalent electromotive force (EME) of the reactions. For a cell in which n electrons are lost from a reductant in one-half cell per mole of oxidized reductant, and are received by one mole of an oxidant in the second half cell:

$$\Delta Gr = -n\ F\ \Delta\ Eh \quad \text{(Rowell, 1981).}$$

where

ΔGr = change in Gibbs free energy for the reaction in J mol^{-1}

n = number of electrons transferred between the two couples per mole of oxidant and reductant involved.

F = Faraday constant = 23.061 kcal $volt^{-1}$ $equivalent^{-1}$

ΔEh = EMF or voltage difference of both half cells.

In aqueous systems, the intensity of oxidation is limited by the electrochemical potential at which water becomes unstable and releases molecular O_2. Similarly, the potential at which molecular hydrogen is released from water represents the lower limit in an aqueous system. In soil and sediment-water systems, many redox couples are usually present and most are not chemically reactive with others. Electrodes used to measure redox potential are not specific for a single redox couple unless it is present at a relatively great concentration. Thus, the platinum electrode responds to the electrochemical potentials of all redox couples present. The resulting measurement represents a weighed average of the potentials contributed by each of the redox couples present in the system (Bohn, 1968). Laitinen and Harris (1965) described how several redox couples, each having greatly different potentials as separate redox systems, may be added together to produce a composite or mixed potential which is several hundred millivolts from the potential of the individual couples.

Although a knowledge of the critical redox potential at which the organic redox systems become unstable and are reduced provides valuable information, it provides no indication of the total capacity of the system to accept electrons and thereby support respiration. For this reason, it is essential that an understanding of the capacity factor in redox reactions in soils be obtained.

The capacity factor of the various redox systems will vary from one soil to another. In general, the amount of O_2 in the soil at the time of flooding of a well drained soil is very low, consisting of that trapped in the air spaces plus that dissolved in the water occupying

the pore space. The amount of NO_3^- present at flooding is likely to be more variable than O_2 but is usually only a few parts per million. Reducible Mn oxides are present in much greater concentrations, with most soils having less than 100 ppm reducible Mn while others may have over ten times as much.

A system that is relatively resistant to changes in redox potential upon small additions of a reductant or oxidant is said to be well poised. Poise is a useful concept in understanding potential measurements and behavior. Poise increases with the total concentration of oxidant plus reductant, and for a fixed total concentration, it is maximum when the ratio of oxidant to reductant is 1 (Ponnamperuma, 1972). Reduced soils and sediments are generally well poised as a result of the presence of relatively great concentrations of soluble iron and perhaps Mn (Gambrell and Patrick, 1978).

1.3 Sequential Reduction

It has been found that the reduction of a waterlogged soil proceeds roughly in the sequence predicted by thermodynamics (Ponnamperuma, 1955; Ponnamperuma and Castro, 1964; Takai and Kamura, 1966; Turner and Patrick, 1968). The same sequence is observed in the vertical distribution of redox components in a well eutrophied lake and in the succession of redox reactions in anaerobic batch digesters (Patrick and DeLaune, 1972).

As stated above, O_2 is the first soil component to be reduced and it disappears within a day after submergence. After the depletion of O_2, the need for electron acceptors by facultative and obligate anaerobic microorganisms results in the reduction of several oxidized soil components. The affinity for electrons of these components depends upon their electron bonding energies (Patrick and Reddy, 1977). In the presence of a readily available energy source, microbes utilize several of the oxidized soil components and reduce the oxidation number of the oxidized atom. Some of these soil components are reduced sequentially; that is, all of the oxidized components of one system will be reduced

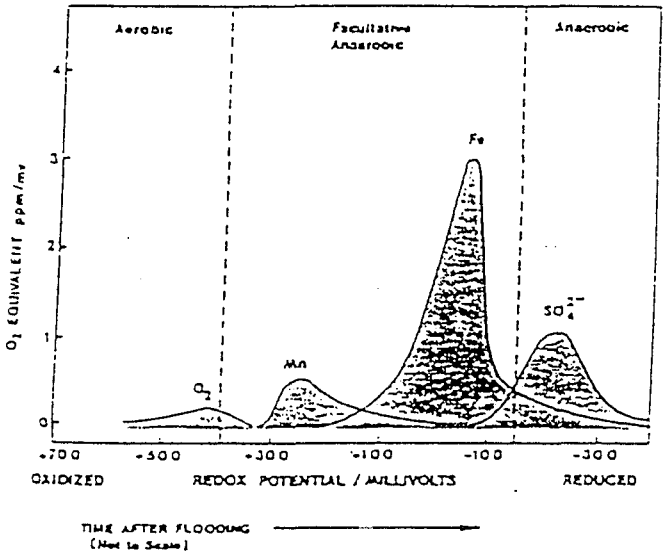

Fig. 1. The capacity of different redox systems to accept electrons decomposing organic matter on the basis of oxygen equivalents. (Reddy et al., 1986).

before any of the oxidized components of another system begin to be reduced. Others overlap during reduction. The general sequence of reduction is shown in Figure 1, which is based on the work of several investigators (Takai and Kamura, 1966; Turner and Patrick, 1968; Connell and Patrick, 1968; and Martens and Berner, 1974).

As O_2 reduction to water comes to an end, NO_3^- is utilized by facultative anaerobiosis. This process is called NO_3^- respiration or dissimilatory NO_3^- reduction. Nitrate reduction begins before complete removal of O_2 (350mV at pH 7) but the removal of all of the NO_3^- cannot be achieved until ali O_2 disappears (Mortimer, 1941; Skerman and MacRae, 1957; Bremner and Shaw, 1958; Turner and Patrick, 1968). Dissimilatory NO_3^- reduction to gaseous end products is commonly known as denitrification. Detailed review of this process has been reported by several investigators (Delwiche and Bryan, 1976; Khalid et al., 1977; Stouthamer et al. 1980, Payne 1973, 1981, Firestone 1982). Several researchers (Keeney et al., 1971, Koike and Hattori, 1978, and Sorenson, 1978), have shown that dissimilatory reduction of NO3- to NH4+ can potentially occur in systems which are anaerobic for long periods, such as rice paddy soils. In soils where temporary anaerobic conditions exist and in soils that are less extensively reduced, denitrification can be the major pathway of removing NO3-. In both pathways, NO_3^- is primarily used as the terminal acceptor during the oxidation of organic matter. Nitrate reduction to N_2 occurs at the values of 200 to 300 mV (Patrick, 1960) while NO_3^- reduction to NH_4^+ occurs at Eh values of less than -100 mV (Buresh and Patrick, 1981). In the reduction sequence, Mn reduction occurs at about the same time or shortly after NO_3^- reduction, with manganic forms being reduced to the more soluble manganous form as the redox potential decreases to about 299 mV (Turner and Patrick, 1972; Gotoh and Patrick, 1972). However, the influence of Mn is weaker than that of NO_3^- because it is insoluble in water and is used as an electron acceptor in respiration by only a limited number of bacteria. Native or added MnO_2 retards the decrease in redox potential of flooded soils and prevents the buildup of great concentration of Fe^{2+} and other reduction

products (Ponnamperuma and Castro, 1964; Ponnamperuma et al., 1965). Ferric iron is reported to be stable until redox potential decreases to around 120 mV (Bohn, 1971; Patrick, 1964).

1.4 Soil pH

Soil reaction is an indicator of the degree of acidity or alkalinity of the soil. To characterize these conditions the term pH is used. The concept of pH was introduced by Sorensen in 1909 and is defined as

$$pH = -\log[H^+] = \log [1/H^+] \quad \text{(Garrels and Christ, 1965)}$$

where $[H^+]$ = activity of H^+ ions.

The determination of soil pH is accomplished by the use of an electromotive force cell that consists of a glass electrode dipping in a test solution together with a reference electrode to complete the circuit.

The glass electrode is a specific type of membrane electrode. It is made of sodium-calcium-silicate glass bulb containing dilute HCl. The Ag-AgCl with 0.1 M HCl serves as the electrode with fixed potential (0.281 V). When the bulb is immersed in a solution, an electric potential is developed between the inner and outer solutions that is proportional to the logarithm of $[H^+]$ in the external solution. Moreover the glass membrane is permeable only to H^+ except under high levels of Na^+ (>1000 mg/L).

The principle of the glass membrane is that the electrochemical potential across the membrane is linearly proportional to the difference in pH of the two solutions in contact with the membrane and can be measured if suitably amplified. If the solute is an ion such as H^+, which unequivalently distributes across a H^+ selective membrane, two potential gradients are involved; the chemical potential gradient which depends on the concentration distribution, and the electrical potential gradient which depends on charge distribution. These forces acting on the H^+ ions create the combined effect that is called the

electrochemical potential gradient. The potential across the glass membrane can be closely calibrated to the approximate value of the H^+ activities.

The chief requisite of the reference electrode is to provide a constant potential at a given temperature. A saturated calomel electrode which is a Hg-HgCl electrode serves this function. The potential of the calomel electrode depends on the concentration of KCl

0.1 M KCl calomel : E = 0.336 V

0.01 M KCl calomel : E°= 0.280 V

Saturated-KCl calomel E = 0.244 V

for 0.1 M HCl Ag-AgCl : E = 0.281 V

Although the KCl-saturated calomel is more sensitive to temperature, it is used by many researchers, because it is easy to maintain. Electrical connection with the test solution is usually established through either a ground-glass joint (sleeve-type) or asbestos fiber projecting through a small hole in the base of the electrode stem. A single probe combination glass-calomel electrode is popular in laboratories. The narrow diameter of the probe permits its use with volume of only a few milliliters. Since an "asymmetric potential" develops across the glass membrane, even when solutions of the same H+ ion activity are on two sides, the meter must be calibrated with a standard buffer. When the two half-cells are immersed in a solution, an electric potential is developed and according to Garrels and Christ (1965) the cell may be written:

Glass electrode, sol. x|KCl(sat.), Hg_2Cl_2(s); Hg(l).

The vertical line drawn between sol. x and KCl (sat.) denotes a "liquid junction potential" a potential which is formed at the interface of two different solutions, i.e., solutions of different electrolytes, or solutions of different concentrations of the same

electrolyte. The saturated KCl bridge helps to minimize this potential. Potassium and chlorine ions are used because their ions mobilities are about the same.

$$K^+ = 7.62 \times 10^{-4} \text{ cm}^2/\text{volt-sec}$$

$$Cl^- = 7.91 \times 10^{-4} \text{ cm}^2/\text{volt-sec}$$

$$H^+ = 36.30 \times 10^{-4} \text{ cm}^2/\text{volt-sec}$$

If something happens to impede the movement of either K^+ or Cl^-, a junction potential is set up. The clay particles may cause this situation. Salts like KCl and $CaCl_2$ decrease junction potential and increase the stability of measurement.

1.5 Changes in pH in Flooded Soils

Within a few weeks after flooding the pH of acid soils increases and the pH of calcareous soils decreases (Ponnamperuma et al., 1966, Ponnamperuma, 1984). Thus the pH of most acid and alkaline soils converge between 6 and 7 after flooding.

When applying the thermodynamic approach is applied to the soil system (Ponnamperuma, 1967; Ponnamperuma et al., 1969) the changes in pH in flooded soils is regulated fundamentally by four systems.

1. $Fe_3(OH)_8$-CO_2-H_2O in ferruginous soils
2. $MnCO_3$-CO_2-H_2O in manganiferrous soils
3. $CaCO_3$-CO_2-H_2O in calcareous soils
4. Na_2CO_3-CO_2-H_2O in sodic soils

In the first two systems, oxidation-reduction control the increase of pH and its stabilization at 6.5-7.0, particularly in acid soils. The last two systems control the decrease of pH in alkaline soils.

Since most soils contain more Fe(III) oxide hydrates than any other oxidant, the increase in pH of acid soils is largely due to the reduction of Fe(III) to Fe(II). It can be described for most acid mineral soils by the following equations (Ponnamperuma, 1984).

$$Eh = 1.06 - 0.059 \log Fe^{2+} - 0.177\ pH$$

or

$$pE = 17.87 + pFe^{2+} + 3\ pH$$

Rowell (1981) observed that during the reduction process in a flooded soil, protons and electrons are removed from the system and the pH will rise. Among the couples prevalent in soils, $Fe(OH)_3$-Fe^{2+}, MnO_2-Mn^{2+} and SO_4^{2-}--H_2S use protons in excess of electrons, and so cause a rise in pH.

The decrease in pH of sodic and calcareous soils and the check on the pH rise of acid soils are the results of accumulation of CO_2. Ponnamperuma et al. (1969) noted the following relationship:

Sodic soil	$pH = 6.4 - 1.00 \log P_{CO_2}$
Calcareous soil	$pH = 6.1 - 0.66 \log P_{CO_2}$
Neutral	$pH = 6.1 - 0.64 \log P_{CO_2}$
Ferruginous soil	$pH = 6.1 - 0.57 \log P_{CO_2}$

In soils low in both Fe and Mn, CO_2 tension may be the dominant factor in controlling pH of flooded soils (Ponnamperuma, 1965). The pH of alkaline soil is highly sensitive to changes in the partial pressure of CO_2 (Whitney and Gardener, 1943, Ponnamperuma et al , 1966). It was noted that the reduction of SO_4^{2-} to S^{2-} contributes to raising pH of flooded soils at lower redox potentials (van Breeman, 1975). Drainage and subsequent oxidation of flooded soils reverses these pH changes (Ponnamperuma, 1972), thereby decreasing pH of acid soils and increasing pH of sodic and calcareous soils. An initial decrease in pH may occur upon flooding even in acid soils due to accelerated CO_2 production by aerobic bacteria.

The rate and degree of pH changes depend on soil properties. Organic matter and reducible Fe largely determine the pH changes in acid soils. If acid soils are low in organic

matter or active Fe, or high in acid reserves such as in acid sulfate soils, they may not attain pH 6.0 even after months of submergence.

1.6 Effect of pH Changes and Redox Potential

Patrick and Reddy (1978) observed that the effect of redox potential and pH on the reactions, and specifically on the final chemical equilibria of a flooded soil, is much greater when these parameters are acting together than when acting alone. Microbial reduction processes, as distinguished from chemical processes, are favored by a near neutral pH. For example, Moraghan and Patrick (1974) found that ferric iron reduction in a submerged soil at pH 7 proceeded faster than at pH 5. However, the final soluble and exchangeable Fe^{2+} concentration of a reduced flooded soil was always much greater at pH 5 than at pH 7 (Gotoh and Patrick, 1974). The reason for this apparent discrepancy is that reduced Mn and Fe produced microbially at near neutral pH rapidly undergo secondary reactions and are precipitated as various oxide and hydroxide forms, whereas at low pH the reduced cations remain in solution or on the exchange complex. This effect of low pH in increasing the solubility can be predicted from the reduction equations for Mn and Fe oxide (Patrick and Reddy, 1978).

$$MnO_2 + 4H^+ + 2e = Mn^{2+} + 2H_2O$$

$$Fe_2O_3 + 6H^+ 2e = 2Fe^{2+} + 3H_2O$$

For nonmetallic redox systems such as NO_3^-, NO_2^-, and SO_4^{2-} there is no beneficial effect of low pH in combination with reducing conditions on reduction of the oxide forms. Both denitrification (Wijler and Delwiche, 1954; Nommik, 1956; Bremner and Shaw, 1958) and SO_4^{2-} reduction (Zobell, 1958; Cappenberg, 1975) are inhibited by low pH.

2. Nitrogen Fixation

The atmospheric N_2 is virtually inexhaustible and constitutes 80% of the atmosphere. A molecule of N is made up of two atoms of N. It is relatively inert because these two atoms are joined by a triple bond and a large quantity of energy is required to break the

bond. In the Claude-Harber process, atmospheric N_2 is combined with H_2 at high temperature (400-500°C), and pressure (200-1000 atm) in the presence of a catalyst containing Fe to form NH_3.

N can be fixed biologically by a few genera of prokaryotic organisms that contain genetic information to sythesize the enzyme nitrogenase. A schematic classification of N_2-fixing organisms is shown in Figure 2. In contrast to the industrial process of N_2 fixation in biological fixations nitrogenase catalyses the conversion of N_2 to NH_3 under mild temperature and normal atmospheric pressures.

$$N{\equiv}N + 3H_2 = 2NH_3$$

The N_2-fixing organism extracts the H^+ from glucose (the principal carbohydrate product from photosynthesis) in the case of autotrophs or from any other energy source in the case of heterotrophs. Actually, it is only the electrons which are actively transferred since there are plenty of protons, or H^+ nuclei in the aqueous medium of the cell. This reaction is an energy consuming redox reaction in which N is reduced and the organic matter is oxidized. It is estimated that 12 to 24 ATP molecules are required for the conversion of one molecule of N_2 into two molecules of ammonia. Not all of this energy goes for the breaking of the triple bond, but some part of it is diverted to competing reactions bringing about the evolution of H_2.

Nitrogenase consists of two soluble proteins labeled Component I and Component II. The average molecular weight of Component I is 220,000. Component I has four subunits, each a single strain of amino acids. It contains 24 Fe atoms and 2 Mo atoms. The average molecular weight of Component II is 55,000. It has two protein subunits and contains four Fe atoms, but no Mo (Brill, 1977). In order to function, nitrogenase needs ATP. ATP is hydrolyzed to ADP and the energy released is used in the fixation process. Metal Mg is also known to be a crucial ingredient for nitrogenase. However, the exact role of this metal is not known. Brill (1977) summarized the N biochemistry. The enzyme Component II receives an electron from an electron transport system external to the

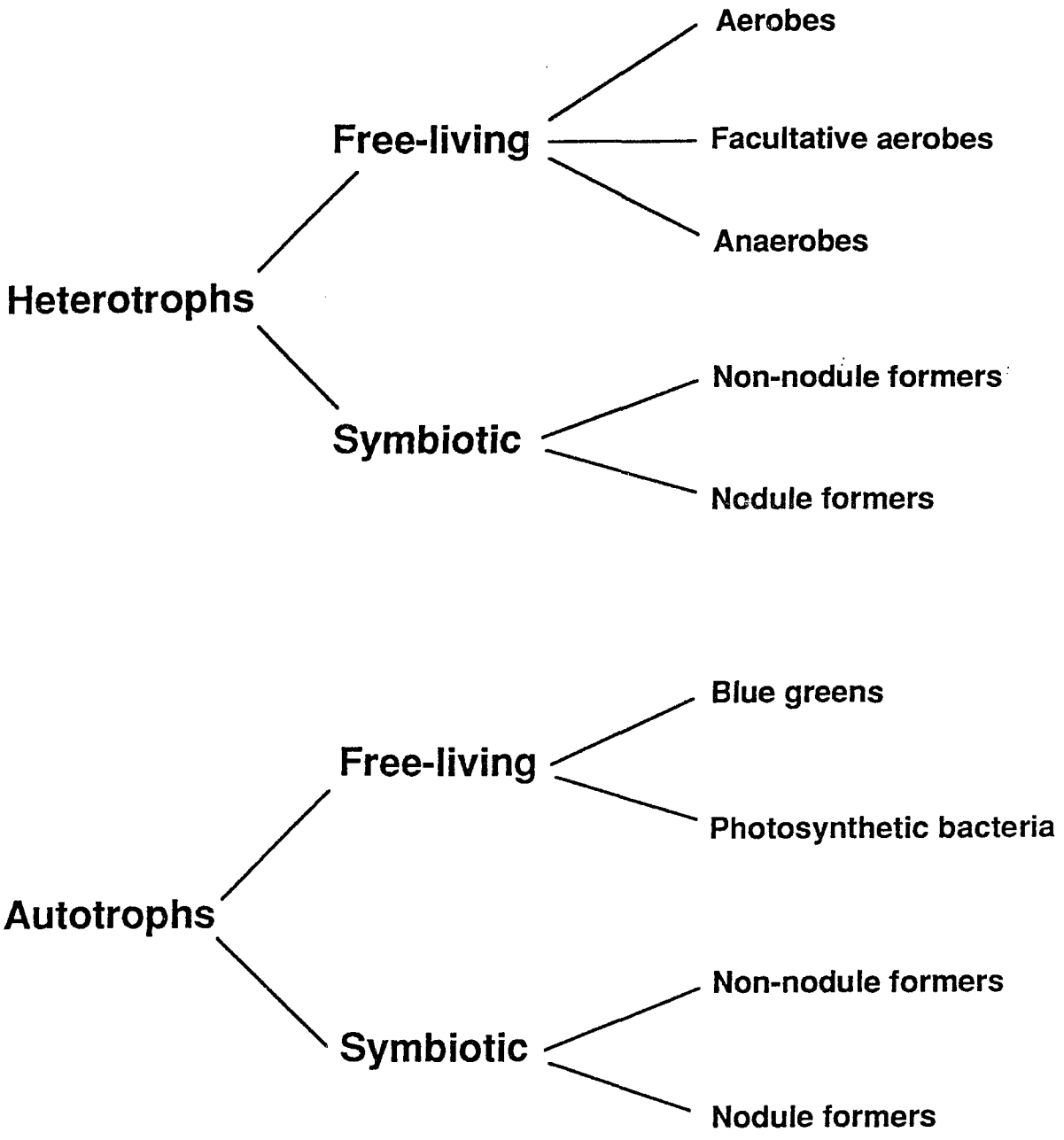

Figure 2. Schematic diagram of some representative N_2 fixers. (Watanabe, 1978; Harelka et al., 1982).

enzyme. Component II passes the electron to Component I which in turn passes it to the N_2 molecule for the formation of NH_3 and other N compounds. Rate of fixation is controlled by another enzyme:glutamine synthetase. This occurs by feedback inhibition from several of the ultimate products of amimo acid synthesis. Both proteins of nitrogenase are extremely O_2 labile. This does not pose a problem for the anaerobic N_2 fixers, the aerobes need a special mechanism for the protection of the enzyme. Such a mechanism, however, decreases the efficiency of N_2 fixation because an increasing amount of C is used to produce a given amount of fixed N_2 (Havelka et al. 1982).

2.1 Factors Affecting Nitrogen Fixation

2.1.1 Energy Source

Content of easily decomposable organic substances appears to be the primary factor controlling heterotrophic N_2 fixation in most soils (Hanson, 1977; Wada et al., 1978; Stewart, 1979; Yamaguchi, 1979). In rice culture, organic C is supplied by living rice plants or by crop residues. The energy source may come from root exudates or directly from root tissues.

Many C substrates, including glucose (Okafor and MacRae, 1973; Jones, 1974), sucrose (Kurn and Brezonick, 1971; Marsho et al., 1975), and rice straw (Rao, 1976; Matsuguchi, 1977; Yoneyama et al., 1977; Reddy and Patrick, 1979; Charyulu and Rao, 1981) have been shown to stimulate N_2 fixation in soils and sediments. N_2 fixation was also enhanced by succinate, acetate, butyrate, and n-propanol, but ethanol, lignin and paraffin were ineffective (Rao, 1978). These differences were attributed to either fluctuation in active microorganisms or the availability of breakdown products and substrates. The extent of N gain is related to quantity of C source added and the prevailing temperature (Fehr et al., 1972). Araragi and Tangcham (1979) noted that addition of rice straw to paddy soil can increase the population of *Azotobacter*. Watanabe and Roger (1984) reported a multiple of 10^5 times increase in photosynthetic bacterial populations

after surface application of 4 tons of straw per hectare in an experiment conducted at the International Rice Research Institute (IRRI). Matsuguchi et al. (1975) in a survey of rice soils in Thailand observed that soils rich in organic matter generally supported higher populations of heterotrophic N_2-fixing bacteria. Rao (1976) reported 1 to 7 mg N_2 fixed per gram of added rice straw. From Charyulu and Rao's data, N_2 fixed per gram of added rice straw (5g/kg soil) in flooded soil ranged from 0 (acid saline soil) to 1.6 kg/ha (acid sulfate) soil. Reddy and Patrick (1979) suggested that N_2 fixation due to incorporation of N poor crop residues may be due to addition of an energy source. Because the active species must compete with other populations for the energy source, the more competitive the active organisms, the greater will be the N gains. Addition of organic matter non-symbiotic N_2 fixation (Watanabe and Brotonegoro, 1981; Charyulu and Rao, 1981). Rao (1976) and Yoneyama et al. (1977) observed retarded fixation in waterlogged soils amended with 2% rice straw as compared to lower levels of amendment. They have speculated that compounds toxic to microorganisms could have formed during the decomposition of large quantities of straw. However, Rice and Paul (1971) reported asymbiotic N_2 fixation in waterlogged soil amended with 20% ground wheat straw. They related the N_2 fixation to the number of N_2-fixing clostridia.

In the rhizosphere of rice as in any other rhizosphere, energy yielding compounds come from three sources: root exudates, root lysates and root litter (Dommergues and Rinaudo, 1979). At harvest stage 3.2 and 6.7% of the total ^{14}C assimilated was exudated from rice varieties IR22 and IR8 roots and remained in the soil and water (IRRI, 1973). These data indicate that exudation, alone, is not an important source of energy for rhizosphere N_2 fixers. Since root exudation depends in part upon light intensity, it is not surprising that shading rice seedlings dramatically decreases rhizospheric N_2 fixation (Dommergues, 1972). Root residues rich in carbohydrates with great molecular weight, such as cellulose cannot be used directly by the N_2-fixers. These residues must be transformed to simpler intermediates by associated rhizospheric microoganisms to serve as

substrates for N_2 fixers. Knowles (1977) defined efficiency of N_2 fixation as mg N_2 fixed per gram of carbohydrate consumed. To fix 1 mole of N_2, anaerobic heterotrophic N_2-fixers require 8-10 moles of glucose, aerobic heterotrophic N_2-fixers 3-4 moles, and approximately 1 mole for *Rhizobium sp.* He concluded that fixation by heterotrophs is generally low. However, he indicated that growth factors, soil extract, colloidal clay, anaerobiosis, and low concentrations of energy yielding substrates may increase the efficiency of N_2 fixation by 2 to 3 times, compared with values obtained on the basis of pure culture.

2.1.2 Redox Potential

Many reports show that heterotrophic N_2 fixation is more active in flooded or anaerobic soils than in aerobic soils (Chang and Knowles, 1965; Brouzes and Knowles, 1969; Buresh et al., 1980; Yoshida and Brotenegoro, 1981). Yoneyama et al. (1977) reported that lower Eh values (less than -200mV) favor N_2 fixation in flooded soil amended with straw. Matsuguchi (1979) and Panichsakpatano et al. (1979) also reported that a decrease of Eh up to -300 mV stimulated N_2 fixation in the reduced plow layer soil. DeLaune et al. (1978) found more N_2-fixing activity in a salt marsh soil at -250 mV near pH 7 than under more oxidizing conditions at lower pH values. Trolldenier (1977) reported that redox potential influenced nitrogenase activity on the roots of rice grown in nutrient solution. Nitrogen-fixing activity increased to a maximum value with decreasing redox potential and declined rapidly with further decreases in redox potential. Wada et al. (1978) and Panichsakpatana et al. (1979) in field investigations found that the development of reduced conditions in the soil is the second most important factor after the availability of an energy source. They noted that N_2 fixation is highly correlated with Eh and Fe^{2+} content. Using an incubation technique Yoneyama et al. (1977) studied the N_2 fixation in three Philippines rice soils at an Eh range of 0 to -300mV. They observed the greatest fixation in the -200 to -260 mV range.

The aerobic-anaerobic interface in flooded soils was found to be an active site of N_2-fixation (Magdoff and Bouldin, 1970; Rice and Paul, 1971). Magdoff and Bouldin suggested that substances formed in the anaerobic zone diffuse up to the aerobic zone and support N_2-fixation by aerobes. However, Rice and Paul (1972) reported that substances formed from straw in the aerobic zone support N_2-fixation by anaerobes. In flooded rice fields, both mechanisms occur depending on the type of organic substances and predominant bacteria. Moreover, anaerobic decomposition of rice straw and cellulose results in the accumulation of organic acids and alcohols that have been shown to stimulate N_2-fixation under submerged conditions (Rao, 1978).

2.1.3 Soil Reaction

The prevailing pH has a profound influence on the abundance of the active N_2-fixing bacteria. *Azobacter* is known to be very sensitive to low pH. Despite the wide distribution of this genus, as a rule, its members are very rare in environments less than pH 6. Unlike these bacteria, *Beijerinchia* species can develop and fix N_2 from pH 3 to 9. The Acid tolerance of the *Clostridium* falls between *Azobacter* and *Beijerinchia* (Alexander, 1977).

Matsuguchi (1979) found that the abundance of heterotrophic bacteria was positively correlated with soil pH. In flooded soil, pH is seldom a problem because the near neutrality of these soils due to waterlogging. However, Matsuguchi et al. (1976) reported that application of 113 kg P_2O_5/ha and 5.7 t/ha of lime improved the growth of both heterotrophic and phototrophic N_2 fixers and their activity, in rice fields of the Central Plain of Thailand.

Yoneyama et al. (1977) observed the greatest N_2 fixation at pH 7.1 to 7.5. Wada et al. (1978) and Panichsakpatana et al. (1979) found a correlation between Japanese rice soils and soil pH. Controlled pH and redox potential incubations of salt marsh sediment by Casselman (1979) revealed that N_2 fixation was greater at pH 6.5 than at either 5.0 or 8.0.

Van Berkum and Sloger (1982) noted the greatest N_2 fixation over a pH range of 5.8 to7.5 with rice grown in water culture.

2.1.4 Soil Oxygen and Water Regime

Because of the susceptibility of nitrogenase to O_2, it is obvious that N_2 fixation is influenced by soil aeration. N_2 fixation has been shown to proceed more actively under conditions of poor rather than normal aeration. Flooded soil conditions in rice fields favor active N_2 fixation by autotrophic and heterotrophic N_2-fixing organisms (Rice et al., 1967; Rinaudo et al., 1971; Kobayashi and Hague, 1971; Dommergues et al., 1973; Rao et al., 1973; Yoshida and Acanjas, 1973).

Aerobic N_2 fixers such as *Azobacter* require O_2 for metabolism; however, *Azobacter* has been shown to fix N_2 most efficiently at low O_2 tensions (Stewart, 1969). Subatmospheric O_2 tension appears to favor proliferation of aerobic N_2-fixing bacteria. Trolldenier (1977) found that an intermediate O_2 level of 3% was more favorable for N_2 fixation in the rice rhizosphere than either 21% O_2 or O_2 free-conditions. Rao (1976) noted that at low moisture contents (24-35%) N_2 fixation was negligible and that N_2 fixation was considerably enhanced under flooded conditions. He also observed that incubation of flooded soils under an atmosphere further accelerated N_2-fixing capacity. The activity increased progressively for 31-40 days and then declined to significant rates at 57 days. Charyulu and Rao (1979) reported that soil submergence favored N_2 fixation irrespective of the properties of the soil studied. Postgate (1974) and Yates (1977) in reviewing the nitrogenase inhibition by O_2 noted the mechanisms to control access of O_2. In effect, some heterotrophic bacteria, such as *Azobacter* increase their respiratory coefficients to protect the N_2-fixing enzyme. In some organisms, the enzyme is isolated in O_2-free compartments. In soil, water content does not directly affect N_2 fixation but controls it by affecting the rates of gas exchanges, especially the O_2 content of the soil atmosphere and soil solution. Therefore, the effect of O_2 and that of the water regime on

the N_2 fixation cannot be dissociated in the field (Dommergues and Rinaudo, 1979). Rao (1976) noted that greater N_2 fixation under flooded conditions could be due to the provision of adequate moisture, nutrient supply, and favorable aeration conditions for the development of N_2-fixing organisms.

2.1.5 Combined N

The inhibition of N_2 fixation by inorganic N is well documented. However, the results in the literature are controversial, they indicate that inhibition of N_2 fixation by inorganic N does not always occur and that the inhibitory effect is dependent upon the amount of inorganic N and other factors in the soil, such as the N_2-fixing microflora. Daesch and Mortenson (1972), and Tubb and Postgate (1973), indicated that inorganic N acts as an inhibitor through depression of the N_2-fixing enzyme biosynthesis.

Matsuguchi (1979) found an inhibitory effect on the phototrophic N_2-fixing microflora and a somewhat stimulative effect on the heterotrophic N_2 fixing microflora located in the lower plow layer (1-10 cm). He found that in the presence of straw, the stimulative effect induced by inorganic N application amounted to 23% in the 3-10 cm plow layer and 43% in absence of rice straw. However, in a pot experiment, Kalininskaya et al. (1973) found that N application (50 kg/ha) stimulated N_2 fixation in the rice-soil system amended with straw (5 t/ha) and adversely affected it in the system without straw. Trolldenier (1977) showed that application of 100 kg/ha of N at transplanting and 40 kg/ha two months later stimulated N_2 fixation of rice-soil systems, presumably because of the rapid decrease in organic N concentration. This stimulative effect of inorganic N on N_2 fixation confirms the importance of heterotrophic N_2-fixing microflora in the lower plow layer. Rao (1976) did not find complete suppression of the nitrogenase activity at concentrations as great as 160 to 320 ppm N in wetland rice soils. The inhibition of the activity of the enzyme due to fertilization might be only temporarily due to the fact that rice plants can rapidly assimilate soil NH_4^+ (Trolldenier, 1977). Jurgersen (1973) speculated

that added fertilizer might stimulate N_2 fixation indirectly through enhancement of greater plant production which subsequently can result in a greater supply of root exudate and residues for heterotrophic fixers. Some minimum concentrations of combined N which suppressed nitrogenase activity are 26-168 ppm NO_3^-N (Delwiche and Nijler, 1956) 290-500 ppm NH_4^+-N (Huser, 1965) and 100 ppm NH_4^+-N (Spiff and Odu, 1972; Yoshida et al., 1972). Balandreau et al. (1974), however, reported that as little as 40 ppm NH_4^+-N partially inhibited activity in a rice rhizosphere. Drozd et al. (1972) noted that the concentration of inorganic N required for suppression in these reports was roughly proportional to the concentration of added carbohydrate.

Charyulu and Rao (1979) investigated the N_2-fixing activity in a laterite rice soil of India at 2 water regimes (flooded and non-flooded) and 4 concentrations of N as $(NH_4)_2SO_4$. They found that the inhibition of nitrogenase was greatest at 60 and 20 ppm N under flooded and non-flooded conditions, respectively. They concluded that the degree of inhibition of N_2 fixation by $(NH_4)_2SO_4$ depends on the soil properties, water treatments, and the concentrations.

2.2 Comparison of Acetylene Reduction and $^{15}N_2$ Methodologies

An extensive review of the acetylene reduction assay (ARA) and ^{15}N techniques has been published by Casselman (1979), and Buresh et al. (1980). N_2 fixation is currently measured by the acetylene reduction technique and by direct reduction of ^{15}N-enriched N gas. The former method is based on the non selectivity of nitrogenase for acetylene and N gas as enzyme substrate. The nitrogenase system apparently functions in similar fashion for each substrate reduced. The assay is based on the similarity in the rate of electron activation and transfer to either N_2 or C_2H_2 as substrate assuming that

$$N_2 + 6H^+ \xrightarrow{6e^-} 2NH_3$$

and

$$C_2H_2 + 2H^+ + 2e^- \rightarrow C_2H_4$$

giving a theoretical ratio of

$$N_2 = 3C_2H_2$$

The ARA entails determination of the microbial formation of ethylene from acetylene. Ethylene produced is measured by a gas chromatograph. The ^{15}N method is, however, less sensitive than the ARA and it requires expensive instrumentation.

The ARA cannot be used as a quantitative tool unless $^{15}N_2$ incorporation experiments are made in identical conditions and experimental ratio of N fixed to reduced C_2H_2 is determined. Reported ratios for different systems range from 3.0 to 6.9 for soils, but values up to 25 have been reported in soils (Hardy et al,. 1973). Because ARA is more sensitive than the $^{15}N_2$ incorporation technique it is difficult to utilized both methods for the same period, particularly when N_2-fixing activity is low. The ARA is, however, useful in comparative studies. In a recent review, Watanabe and Roger (1984) presented the drawbacks of both methods.

1. Acetylene diffusion of formed C_2H_4 is slow. To introduce C_2H_2 into the system, evacuation of the gas phase and mechanical disturbance are produced. Mechanical disturbance is also necessary to recover C_2H_4 from the soil. Because C_2H_2 is more soluble than N_2 in water, the theoretical conversion factor of 3 may not apply and the actual ratio should be found by using $^{15}N_2$ or the Kjeldahl method.
2. Acetylene inhibits N_2-fixing activity of CH_4-oxidizing bacteria, therefore N_2-fixation cannot be determined. Methane oxidation occurs in paddy soils.
3. Acetylene is decomposed under anaerobic conditions. Thus, prolonged incubation under anaerobic conditions leads to great ARA values due to the stimulation of N_2-fixation by microorganisms that probably use the decomposition products of C_2H_2.

4. A great number of replicates (more than 6) and composite soil samples are needed for accuracy. In addition, logarithmic transformation of data is necessary for statistical analysis.
5. The ^{15}N technique cannot be used in the field during the growth cycle of rice because of its great cost, point time measurement, and sophisticated apparatus to control environmental conditions in the closed chambers. Besides, the results of laboratory experiments on photodependent N_2 fixation are often expressed per unit weight of soil.

2.3 Factors Regulating the Aquatic Acetylene Reduction Technique

Ethylene is quite soluble in water. Compared with CH_4, O_2 and N_2, it is respectively 3.7, 3.9 and 8.1 times more soluble than these other gases (Flett et al., 1976). The percentage transfer of C_2H_4 is inversely proportional to the percentage of aqueous phase and directly proportional to temperature. Matsuguchi et al. (1977) reported that the C_2H_2 reduction assay increased with evacuation pressure and reached the maximum when evacuated under 0.01 atm. They also noted that the amount of C_2H_2 which diffused back to the headspace was increased by extending the duration and the maximum recovery was obtained after 1 min vibration. Flett et al. (1976) urged that samples be shaken immediately upon injection of the C_2H_2 to ensure that the nitrogenase is saturated at the start of incubation period. The amount of C_2H_4 that diffuses from solution into the vapor phase in an equilibrated closed system depends upon the relative amounts of aqueous and vapor phases present and can be estimated from Henry's Law:

$$100X/M = 100/(1+ a\ A/B)$$

where, X=volume of gas diffused from aqueous to vapor phase (ml), M=total volume of the gas initially dissolved in the aqueous phase (ml), a=volume of gas that will dissolve in 1 ml of water at a given temperature under 1 atm of partial pressure (ml), so-called Bunsen absorption coefficient, A=volume of the aqueous phase (ml), and B=volume of the vapor phase (ml).

Matsugushi et al. (1977) suggested 1 min vibration for C_2H_2 remaining in the soil to diffuse back to the headspace. Equilibration occurred in about 20 s in this case, where aqueous and vapor phases were equal in volume. Assay sensitivity can be enhanced simply by increasing the relative proportion of aqueous phase in the incubation bottle rather than by concentrating the sample.

Matsuguchi et al. (1976) compared 3 C_2H_2 concentrations (0.02, 0.05 and 0.1 atm). The ARA markedly increased as the pC_2H_2 became greater. The maximum velocity (V_{max}) and the apparent Michaelis-Menton Constant (Km) were 294 nmol C_2H_4 g soil/hr and pC_2H_2 of 0.036 atm, respectively. The ARA measured at pC_2H_2 of 0.1 atm was 230 nmol C_2H_4/g soil/hr, corresponding to 80% of the V_{max}.

The distribution of biological parameters in nature is frequently not normal, and that in the case of several sets of C_2H_2 reduction assay data there was a log-log relation between mean and variance (Roger et al., 1977). Thus, the commonly used t-test would not be applicable without an appropriate log transformation of the data.

3. Decomposition of Soil Organic Matter

The organic fraction of the soil consists of a vast array of complex substances. These compounds are represented by the various components of organic residues undergoing decomposition and the matabolic by-products of microorganisms utilizing organic residues as a source of energy. These diverse groups include:

1. Proteins and their derivatives (amino acids, amides, amino sugars, nucleoproteins, and purines and pyrimidine bases);
2. Carbohydrates, their decomposition products, and related compounds (mono- and dissaccharides, cellulose, hemicellulose, pectins, pentosans, mannans, polyuronides, urionic acid polymers, organic acids, alcohols, hydrocarbons and aromatic compounds).

3. Lignins, various organic phosphorus compounds, tannins and their various decomposition products (Waksman, 1938; Broadbent, 1953; Kononova, 1961; Stevenson, 1964). In addition to the intermediate products of decomposition and secondary products of microbial synthesis, there also exist in soil the more resistant products of decomposition (Kononova, 1961).

The decomposition of organic matter in soil is generally carried out by a wide group of heterotrophic microorganisms and is accomplished in two primary steps which occur simultaneously. These steps are (1) the breakdown of the freshly added organic substances, and (2) the decomposition of native soil organic matter or humus. Plant and animal residues added to the soil are first broken down to their basic structural units by extracellular enzymes produced by heterotrophs. These units are then oxidized intracellularly by microorganisms in order to derive energy (Tusneem and Patrick 1971).

The nature of the flora involved in organic matter decomposition varies with the chemical composition of the added substrates. Certain microbial groups predominate for a short time, while others maintain high populations for a considerable time. Each individual organism is equipped with a complex of enzymes which permits it to oxidize a fixed array of chemical compounds. If the proper substances are present in an accessible state, the microorganisms will proliferate, provided that it can withstand the competition of other organisms possessing similar enzymatic characteristics. The microorganisms preferentially proliferating on the basic components of the added carbonaceous substances constitute the primary flora. These are followed by a secondary flora growing either on compounds produced by the primary flora or on the dead or living cells of the primary flora (Alexander, 1977). The secondary flora use more complex material, such as polysaccharides where the tertiary flora are capable of metabolizing complex polymers such as lignin, but these are not readily assimilable energy sources and their growth is quite slow (Alexander, 1973).

The amount and composition of the microflora are generally determined by the amount, type, and availability of organic matter. The addition of simple sugar causes rapid

proliferation of bacteria, while starch accelerates the activity of actynomycetes, and cellulose benefits fungal development in particular (Alexander, 1977). Proteins and amino acids, such as blood meal or peptone stimulate spore forming bacilli (Winogradsky, 1924; Mollenhoff et al., 1936).

The anaerobic decomposition of organic matter in waterlogged soils is a considerably modified process and can be differentiated from the aerobic decomposition by two important aspects: first, by its much slower rate, and second, by the nature of the end products formed.

The microbial decomposition of organic matter in a well-drained soil is accomplished by a wide group of microorganisms in which fungi play a prominent role. Respiration by these organisms is associated with great energy release, rapid decomposition of substrate and synthesis of all substances. As a result, most components forming the bulk of the added organic matter quickly disappear as CO_2, while those less susceptible to microbial attack persist. As cell synthesis proceeds, a heavy demand is placed upon the soil nutrients. This is particularly true for N, except when highly nitrogenous materials are added.

Anaerobic decomposition, on the other hand is largely accomplished by anaerobic bacteria. Facultative anaerobiosis will also assist in decomposition as long as alternative electron acceptors are present. Since anaerobic bacteria operate at a much lower energy level, they are much less efficient than anaerobic microflora. Consequently, the process of both decomposition and resynthesis are much slower in a waterlogged soil than in a well-drained soil. This is in agreement with the general observation that a greater accumulation of plant residue occurs in bog and marsh soils.

Tenny and Waksman (1930) conducted a comprehensive study of the decomposition rate of various chemical constituents of a variety of plant material under both aerobic and anaerobic conditions and found that anaerobic decomposition was slower than aerobic decomposition regardless of the type of plant material used. The difference in the rates of

aerobic and anaerobic decomposition was even more pronounced for organic nitrogenase complexes. For corn stalks great in water-soluble substances, including reducing sugars and N compounds, 20% of the total material was lost under anaerobic conditions. Relatively slower rates of decomposition were observed with rye straw, which has a lower content. About 17% of the rye straw decomposed in 66 days and 29% in 143 days in the aerobic system, whereas under anaerobic conditions 7 and 22% were decomposed in 84 and 163 days, respectively. The three principal chemical constituents of plants, cellulose, hemicellulose, and lignins also decomposed much more slowly under anaerobic than under aerobic conditions. A similar decomposition pattern was observed with alfalfa in spite of its relatively great N content. This indicates that even a high N content did not cause rapid decomposition under anaerobic conditions. This is not true only for the total plant material, but also for the individual plant constituents. It is noteworthy that accumulation of proteins was greater under anaerobic conditions than under aerobic conditions in all cases. This may have been due to the more economic use of N, to the small loss of NH_3, or to the lesser decomposition of synthesized protein in the anaerobic system.

Acharya (1935a, b) demonstrated that the decomposition of rice straw was most rapid in aerobic environments, slower under waterlogged conditions, and the least pronounced under complete anaerobiosis. This was equally true for the decay of major plant constituents. He showed that the amount of N required for decomposition of rice straw was greater under aerobic conditions than under anaerobic conditions. Conversely, the net release of inorganic N to the solution phase was greater in the anaerobic than in the aerobic system. Quantitatively, the N release by decomposing rice straw under anaerobic conditions was about 5 to 6 times greater than that released under aerobic conditions. Sircar et al. (1940) presented similar evidence and reported that inorganic N release from decomposing rice straw occurred at a greater C/N ratio under anaerobic conditions. They further noted that a minimum N concentration in rice straw of 1.70 to 1.90% was necessary for net accumulation of mineral N, whereas 0.45 to 0.50% was sufficient under anaerobic

conditions. In a field study in California, Williams et al. (1968) concluded that the N concentration required for the decomposition of rice straw in submerged soils was one-third (0.54 vs. 1.5%) the average concentration of N required for aerobic decomposition of plant residues. Waring and Bremner (1964) observed a more rapid release of inorganic N under aerobic conditions in a number of soils. They showed that in a 2-week period, for every 1 part of inorganic N produced aerobically 1.23 parts were produced under waterlogged conditions.

The second and the most striking difference in the decomposition of organic matter under waterlogged and well drained soil conditions lies in the nature of the end products formed. In a well drained soil, the main end products of decomposition are CO_2, NO_3^-, SO_4^{2-}, water and resistant residues. The waterlogged soil, on the other hand, is generally characterized by the formation of chiefly CO_2, CH_4, H_2, organic acids, NH_3, amines, mercaptans, H_2S, and resistant residues. The metabolic degradation of carbohydrate is probably the same under both aerobic and anaerobic conditions until the formation of pyrivic acid as the final end product of glycolysis. In an aerated soil pyrivic acid enters the Krebs Cycle (Tusneem and Patrick, 1971). This is a common channel not only for the oxidation of the end products of glycolysis but also of the ultimate oxidation of the fatty acids and the carbon skeleton of many amino acids. In the presence of O_2 all these carboxylic acids are converted to CO_2 and water through the mediation of terminal oxidases. In waterlogged soil marked with the virtual absence of O_2, the terminal oxidation is suppressed, resulting in the accumulation of pyrivic acid and reduced nicotinamide-adenine dinucleotide (NADH). The pyrivic acid undergoes various transformations not specific to any organisms, but presenting a common feature of anaerobic fermentation.

Acharya (1935a, b, c) examined in detail the course of anaerobic decomposition of rice straw under waterlogged conditions as compared with the aerobic decomposition. He showed that apparently decomposition proceeded in two distinct phases. The first phase

resulted in the rapid formation of organic acids and the second phase transformed these acids into gaseous products. These products of decomposition were acetic acid, butyric acid, CO_2 and CH_4, and trace amounts of H_2. In the presence of neutralizing agents, over 20 liters of gas containing about 50% CH_4 were obtained from the decomposition of 100 g of straw. He also noted that the strictness of anaerobic conditions caused the amount of CH_4 given off to increase, while CO_2 decreased, and H_2 first increase and then decreased. Yamane and Sato (1961) studied the decomposition of glucose, sucrose, starch, cellulose, gelatine, pectic acid, and lignin. Immediate evolution of H_2 was observed where soluble sugars were added. It was then followed by CH_4 after the reabsorption of H_2 by the soil. The H_2 did not persist more than one day. In about two weeks large amounts of CO_2 and CH_4 were evolved from all but the lignin treatment. In the temperature range 15 to 50°C, the maximum production of gases was observed at 40°C. Below or above this temperature the evolution of gases was greatly suppressed. These studies showed that organic acids rather than sugars constitute the basic substrate for bacterial species that produce methane. The anaerobic C breakdown must therefore be characterized by the formation of organic acids, CH_4, and CO_2 as the major end products. The final end products in the decomposition of protein are usually NH_3, carboxylic acids, amines, mercaptans, and H_2S. The metabolic pathway of protein breakdown is probably the same under both waterlogged and well-drained soils until the formation of amino acids occurs. Under aerobic environments, the process of deamination takes place and NH_4^+ is either held on colloidal particles in exchangeable or non-exchangeable form or is oxidized to NO_3^- through the activities of nitrifiers. Under anaerobic conditions products of deamination and of subsequent decarboxylation may accumulate or be transformed into gaseous products.

3.1 Nitrogen Transformations in Soils

Over 90% of the N in the surface layer of most soils occurs in organic form, with most of the remainder being present as NH_4+ which is held within the lattice structures of

clay minerals and only a small amount (1-2%) appearing in the inorganic fraction which is the immediate plant-available N. Organic N forms the potential reserve of N for the nutrition of plants but it is available to plants only after it has been converted to the inorganic form (Tusnem and Patrick, 1971; Alexander, 1977; Stevenson, 1982).

3.1.1 Inorganic Nitrogen

The inorganic N is chiefly present as NH_4^+ and NO_3^--N. Nitrite may also be present as an intermediate product of nitrification, denitrification, and NO_3^- reduction. The amount of NO_2^- present in soil is generally negligible but may accumulate in neutral or alkaline soils, particularly when fertilized with large amounts of NH_4^+ (Martin et al., 1943; Chapman and Liebig, 1952; Duisberg and Bueher, 1954; Fuller, 1963). Accumulation of NO_2^- may also occur in acid soils treated with anhydrous ammonia (Nommik and Nilsson, 1963) or urea (Soulides and Clark, 1958; Clark et al., 1960; Stephen and Waid, 1963). Hauck and Stephenson (1965) described the NO_2^- accumulation with large granule size, low soil buffer capacity, an alkaline pH of the immediate granule environment and heavy amount of N application. Broadbent et al. (1957), and Aleem and Alexander (1960) speculated that the accumulation of NO_2^- is due to the inhibiting effect of NH_4^+ on the oxidation of nitrite to nitrate by *Nitrobacter* species. Several other forms of inorganic N, such as hydroxylamine, hyponitrous acid, and nitramide, may occur as intermediates in the microbial processes leading to nitrification, N_2 fixation, and denitrification. These compounds are chemically unstable, and hence, are not detected during the course of analysis.

Most of the inorganic N is water soluble or adsorbed on the exchange complex. Approximately 5% of the NH_4^+-N in surface soils may be fixed in the lattice of silicate minerals in non-exchangeable form (Barshad, 1951; Allison et al., 1953; Nommik, 1957; Stojanovic and Broadbent, 1960; Bremner, 1965a). The magnitude of NH_4^+ fixation may be 30% or more in certain soils. Montmorillonite, illite, and vermiculite are the clay

minerals largely responsible for ammonium fixation. The inorganic N constitutes a very dynamic system and undergoes rapid changes in its form and amount.

The inorganic N regime in waterlogged soils is characterized by the accumulation of NH_4^+ and the absence of NO_3^-. The NO_3^- present in soils before waterlogging may be removed by leaching, denitrification, and plant uptake. Some of the NO_3^- could be reduced to NH_3, but the amount reduced is insignificant (De and Sarcar, 1936; Broadbent and Stojanovic, 1952; Wijler and Delwiche, 1954). The NO_2^- levels in waterlogged soil are low. They generally lie in the range of 0-3 ppm (Jassen and Metzgar, 1928; Kapp, 1932; De and Sarcar, 1936; Reed and Sturgis, 1937).

Ammonium N, which constitutes almost completely the inorganic pool of N, undergoes the same reactions in waterlogged soils as in well drained soils, with the exception of nitrification, which is severely restricted. Ammonium can be taken up by the plants, immobilized into cell substances, adsorbed on the clay crystal lattice, and volatilized under alkaline conditions. The degree and rate of these reactions depends on the conditions bringing about the transformations.

3.1.2 Organic Nitrogen

The nature of N-containing organic compounds occurring in soil is not well understood because of the analytical limitations. Organic N is usually determined by measuring total N and subtracting inorganic N (exchangeable and non-exchangeable NH_4^+, NO_2^-, and NO_3^-). Two methods that are widely used for total N determinations are wet oxidation (Kjeldahl) and dry combustion (Dumas). Reviews of the methods have been published by Bremner (1965b), Bremner and Tabatabai (1971), Stevenson (1982).

The methods used for fractionation of organic N in soils have been based on studies involving identification and estimation of the N compounds released by the treatment of soils with hot acids to liberate nitrogenous constituents from clay and organic colloids. These methods are essentially a modification of Hausmann (1899) and Van Slyke (1911-

12, 1915). That part that is not solubilized by acid hydrolysis is referred to as insoluble-N and that recovered by distillation with MgO is NH_3-N. The soluble N not accounted for as NH_3 or known compounds is the hydrolyzable unknown (HUN) fraction. More specific methods of characterizing organic N substances have been developed which permit both determination and isotope-ratio analysis of total N, NH_4^+-N, hexosamine N, serine+threonine N, hydroxy amino acid N, and amino acid N in soil hydrolyzate (Bremner, 1965b).

The main identifiable organic N compounds in soil hydrolyzates are amino acids and amino sugars. Soils contain trace amounts of nucleic acids and other nitrogenous biochemicals, but specialized techniques are required for their separation and identification. Only one-third to one-half of the organic N in most soils can be accounted for in known compounds. The procedure for hydrolyzing the soil has been standardized and many variations in hydrolitic conditions have been employed. The variables include: (1) type and concentration of acid, (2) time and temperature of hydrolysis (3) ratio of acid to soil, and (4) pretreatment (Kowalenko, 1978).

An unusually large amount of the N in soil, usually of the order of 20 to 35%, is recoverd as acid-insoluble-N. At one time it was thought that this fraction was an artifact resulting from the condensation of amino acids with reducing sugars during hydrolysis, but it is now believed that part of this N occurs as a structural component of humic substances. The possibility that artifacts are formed during hydrolysis cannot be dismissed, however, since addition of glucose to soil immediately prior to hydrolysis has been shown to result in increases in the percentage of the soil N as acid insoluble-N and decreases in hydrolyzable amines and amino acid-N (Asami and Hara 1970). The percentage of the soil N recovered in acid insoluble forms can be reduced by pretreating the soil with HF prior to hydrolysis (Cheng et al. 1975). Also, some of the insoluble N can be extracted with a dilute base and subsequently solubilized by acid hydrolysis, thereby reducing the acid insoluble fraction to about 15% of the total soil N.

Another unique feature of N fractionation schemes is that a large portion of the soil N, usually of the order of 20 to 30% for surface soils, is recovered as NH_3. Some of the NH_3 is derived from indigenous fixed NH_4^+, part comes from amino sugars and the amino acids, amides, asparagine and glulamine. It is also known that NH_3 can arise from the breakdown of certain amino acids during hydrolysis. Tryptophan is lost completely; others such as serine and threonine, are destroyed to a lesser extent.

An accounting of all potential sources of NH_3 in soil hydrolyzates shows that the origin of approximately one-half of the NH_3, equivalent to 10 to 20% of the total organic N, is still obscure. Some NH_3 may be derived from complexes formed by fixation reactions. Haider et al. (1965) examined the reaction of proteinaceous substances with phenols in the presence of phenoloxidase enzymes and found that part of the amino acid-N was liberated as NH_3 when the reaction products were subjected to acid hydrolysis.

Part of the N which is solubilized by acid hydrolysis occurs in unknown forms. Greenfield (1972) concluded that most of the N in this fraction occurred as monamino N in arginine, tryptophan, lysine, and prolyne. The monoacid N in these amino acids is not included with the amino acid-N values as determined by the ninhydrin-NH_3 or ninhydrin-CO_2 methods. Goh and Edmeades (1979) concluded that from one-fourth to one-half of the hydrolyzable unknown N in the soils they examined occurred as non-a-amino acid-N.

3.1.3 Distribution of Added Nitrogen in Soils

The advent of the isotopic tracing techniques using ^{15}N to differentiate between the added and soil N, and the subsequent development in the fractionation and determination procedures (Bremner 1965c) made the study of the distribution of added inorganic N of soil N possible.

Several researchers found that a large proportion of the added N in surface soils is converted into the amino acid fraction and a smaller portion goes to amino-sugar components (Bremner, 1955; Sowden and Ivarson, 1959; Stewart et al., 1963; Cheng and

Kurtz, 1963). Bremner (1955) composted plant residues and fertilizer N in the absence of soil. He found that a large fraction of fertilizer N immobilized during the biological decomposition of straw was in the form of bound amino acids and a smaller fraction was in the form of sugars. Sowden and Ivarson (1959) came to similar conclusions in a decomposition study of forest litter. These studies were made in the absence of soil, thus no definite extrapolation of these results could be made to the soil system.

Stewart et al. (1963) studied N transformations in soil incubated with straw and various N fertilizers. They found that most of the N immobilized during the incubation was recovered in the nondistillable acid-soluble N fraction, which is the amino acid fraction. Although this nondistillable fraction constituted only half of the total organic N, ^{15}N studies showed that about three times as much N entered the amino acid fraction as went to the other organic fractions. They noted an inverse relationship between inorganic N and amino acid N. In a lysimeter study involving three soils fertilized with ^{15}N-enriched N and cropped to corn for two consecutive years, Cheng and Kurtz (1963) drew similar conclusions. About 65 % of the added N was recovered as amino acid components and slightly over 10 % was detected in amino acid sugars. These two fractions together with the hydrolyzed ammonium fraction contained over 90 % of the added fertilizer N. The amounts found in soluble and insoluble humin-N fractions were very small (5-10%). The fixation of added NH_4^+ was insignificant (0-1 ppm).

Information on the distribution of added N into the soil organic pool of N under waterlogged conditions is scarce. The soil microbiology group at the IRRI (1965) studied the fate of applied N in submerged soils in order to investigate movement of inorganic N into the organic fractions of soil N and its subsequent release. The hydrolyzed NH_4^+, amino acid fraction, and amino sugars were the most important organic fractions in terms of immobilization. Asami (1971) reported the distribution of labelled immobilized N to be mainly in hydrolyzable ammonium, alpha-amino N and nonhydrolyzable fractions. Tusneem and Patrick (1971), in a waterlogged soil amended with rice straw, found 41% of

immobilized N in the a-amino fraction, compared with 14% in all other fractions combined, with only negligible amounts immobilized in the nonhydrolyzable component of organic matter.

3.1.4 Mineralization and Immobilization of Nitrogen

Of the various changes N can undergo in soil, the mineralization-immobilization cycle is the most continuous, but the two individual processes often achieve opposite goals. Mineralization is the conversion of organic N into inorganic form (NH_4^+). It is carried out by the activities of general purpose heterotrophs which utilize nitrogenous organic compounds as their energy source. Immobilization, is the conversion of inorganic N into organic compounds. The inorganic N is assimilated by microorganisms and metabolized into nitrogenous constituents of their cells. In general, mineralization is always accompanied by immobilization; the two processes tend to counteract each other so far as the production of inorganic N is concerned (Jansson, 1963). Jansson (1953) postulated that the mineralization-immobilization cycle repeats itself, leading towards exhaustion if no fresh organic matter is added from outside the system. When plant residues with a great C:N ratio are used, it is mainly the carbon which is liberated and lost as CO_2 during consecutive cycles through the turnover, while the N is retained in organic form and passes through the cycle many times, until the C:N ratio has become sufficiently reduced to allow for an accumulation of inorganic N. It is at this stage that the term net mineralization of N can be applied (Tusneem and Patrick, 1971). The resulting net effect usually depends on the ratio between the carbonaceous material (energy source) and the N in substances undergoing decomposition (Jansson, 1963). The substances rich in N favor net mineralization and those poor in N favor net immobilization.

To describe the interaction between microbial demand and supply, Alexander (1977) gave a quantification of N immobilization-mineralization. In the process of decomposition, C is released as CO_2 and organic N as NH_4^+. A simultaneous synthesis of additional

microbial cell constituents results in the assimilation of C and N from either the environment or the substrate, depending on the type of microflora involved. Alexander (1977) has developed a formula based on data using the amount of C assimilated and the C:N ratio of the cells synthesized to estimate the N needs for microbial cell syntheses. It is estimated that 5-10% of the substrate C is assimilated and the C:N ratio of cells synthesized 15-30% by actinomycetes, and only 2-5% by anaerobic bacteria. The C content of microbial protoplasm is fairly constant and typically stays within 45-50% of the dry weight, but the N content is variable and depends upon environmental conditions of the system. As an approximation, C:N ratios of 5:1, 10:1, and 5:1 have been proposed for the cellular components of bacteria, fungi and actinomycetes, respectively. It can then be calculated that for the decomposition of 100 units of substrate C, it is necessary to provide 1 to 2, 3 to 4, and 3 to 6 units of N for aerobic bacteria, fungi, and actinomycetes, respectively, while only 0.4 to 1.0 units of N are needed by anaerobic bacteria for the same degree of decomposition. It may be noted that with plant material having 50% C, for each 100 grams of organic matter undergoing decomposition, 0.2 to 0.5, 0.5 to 1.0, 1.5 to 2.0 and 1.5 to 3.0 grams of N are needed by anaerobic bacteria, aerobic bacteria, fungi, and actinomycetes, respectively. From the above figures it is evident that immobilization is greatest for actinomycetes and least for anaerobic bacteria.

Several comprehensive reviews on the aspects on N immobilization-mineralization in well-drained soils have been published by Harmsen and van Schreven (1955), Dubber (1955), Winsor (1958), Jansson (1958, 1963, and 1971), Bartholomew (1965), Drouineau (1965), Allison (1966) and Jansson and Person (1982). Unlike well-drained soils, the information on this aspect of N transformations in anaerobic conditions is meager.

Ammonia is derived from anaerobic deamination of amino acids, degradation of purines, and hydrolysis of urea. Less than 1% comes from NO_3^- reduction (Woldendroop, 1965). The degradation of organic matter which leads to the liberation of

NH_4^+ ions into the solution phase proceeds at a much slower rate in anaerobic environments than in aerobic environments (Tenny and Waksman,1930; Acharya, 1935a and c). However, inorganic N release is larger and faster in anaerobic soils than in aerobic soils (Waring and Bremner, 1964; Broadbent and Reyes, 1971; and Tusneem and Patrick, 1971) because less immobilization of N occurs in an anaerobic media.

The characteristic features of anaerobic bacterial decomposition of organic matter in waterlogged soils comprise: (1) incomplete decomposition of carbohydrate into CH_4, organic acids, H_2 and CO_2 with consequent low energy yield (Alexander, 1977); (2) low energy of fermentation, resulting in the synthesis of fewer microbial cells per unit of C degraded; for example, only 2 to 5% of the substrate C is assimilated by anaerobic bacteria compared with about 30 to 40% assimilation by fungi in aerobic systems (Alexander, 1977); (3) low N requirement of anaerobic metabolism leading to more rapid release of NH_4^+ ions than would ordinarily be expected because of the wide C:N ratio of decomposing plant material and the much slower rate of anaerobic decomposition. In a laboratory study of the decomposition of rice straw under aerobic, partially aerobic (waterlogged) and strictly anaerobic conditions, Acharya (1935c) demonstrated that the rate of decomposition of straw, the N required to decompose 100 grams of material, were greatest under aerobic conditions. Therefore, the net release of inorganic N to the solution phase was greatest in anaerobic and least in aerobic systems. Broadbent and Nakashima (1970) found that the N factor varied with quantity of straw added, the N content of straw, the nature of the soluble N added, the degree of anaerobiosis, and the soil, but in no case was it considerable. The N released to the aqueous extract by rice straw decomposing under anaerobic conditions was about 5 to 6 times greater than that released under aerobic conditions. The waterlogged treatment stood in between these two extremes. Sicar et al. (1940) presented similar results and reported that inorganic N release from decomposing rice straw occurred at a greater C:N ratio under anaerobic conditions. They noted that a minimum concentration of 1.7 to 1.9% was necessary for the aerobic release of N from

decomposing rice straw, whereas only 0.45 to 50% was sufficient under anaerobic conditions. Williams and coworkers (1968), studying the decomposition of rice straw under flooded conditions, concluded that the N requirements for the decomposition of rice straw in flooded soils was one-third (0.54 vs. 1.5%) the average concentration of N required for aerobic decomposition of plant residues.

Mitsui (1954), IRRI (1964) and Tusneem and Patrick (1971) showed the marked influence of C:N ratio on the rate of inorganic N release. The accumulation of inorganic N followed the general order of C:N ratios under both flooded and nonflooded conditions. Further, at wide C:N ratios there was a relatively more rapid release of inorganic N under flooded than nonflooded conditions, particularly during the first three months of incubation. They attributed this to the fact that anaerobic microorganisms predominate under waterlogged conditions and these microbes consume less N as microbial cell constituents than the aerobic microbes. Reddy and Patrick (1984) noted that, under anaerobic conditions, organic residues with C:N ratios lower than 80 favor N mineralization and those greater than 80 favor N immobilization. Under aerobic systems, these processes are balanced at C:N ratios of approximately 23.

Bhuyiyan (1949) and Lopez and Galvez (1958) reported that flooding increased the quantity of NH_4^+ in soil solutions to a maximum level, after which no appreciable change occurred. Patrick and Wyatt (1964) found considerably greater rates of inorganic N release in waterlogged soils than in well drained soils. Waring and Bremner (1961) observed a more rapid rate of net mineralization under waterlogged than aerobic conditions. They noted that in a two week period, for every 1 ppm of inorganic N produced aerobically, 1.23 ppm of NH_4^+-N were produced during incubation under waterlogged conditions.

3.1.5 Effect of pH on Nitrogen Mineralization

Mineralization is influenced by the pH of the environment. All other factors being equal, the production of inorganic N (NH_4^+ plus NO_3^-) is greater in neutral than in acid

environments (Ishaque and Cornfield, 1972), although some soils show little influence of pH on the transformation. Sircar et al. (1940) noted that decomposing organic matter tends to lower the pH of the medium and inhibit the fermentation process under anaerobic conditions unless proper neutralizing agents are employed. The anaerobic bacterial flora operate most efficiently around neutral pH. This may explain why the liming of paddy soils in Japan has sometimes caused an appreciable increase in ammonification (Mitsui, 1954). Nitrogen mineralization under submerged conditions is usually not limited as much as would be expected by adverse pH conditions, however, since submergence tends to drive the pH of both acid and alkaline soils toward the neutral point (Ponnamperuma, 1972) and submerged soils have a narrower range of pH values than do drained soils.

The use of ^{15}N demonstrates that under almost all conditions mineralization and immobilization occur simultaneously. The resulting net effect depends on the ratio between the carbonaceous material and the N in the decomposing substances.

3.1.6 Use of ^{15}N in Soil Nitrogen Studies

Because of the larger supplies of ^{15}N-enriched and ^{15}N -depleted materials at a cost several-fold lower than a decade ago, ^{15}N tracer techniques are common in many laboratories. The use of the stable N isotopes ^{14}N and ^{15}N as tracers is based on the fact that they occur naturally in an almost constant ratio ($^{14}N/^{15}N$=272/1). Naturally occurring N contains about 0.366 atoms % ^{15}N or about 3660 ppm ^{15}N. Addition of material with an unusually high or low concentration of ^{15}N to a system will result in an increase or decrease in ^{15}N concentration in all or part of the system. The change in N isotope ratio in samples obtained from the system permits study of the transformations of the added tracer material. The amount of change in isotope ratio from the background level permits calculation of the extent to which the tracer has interacted with and becomes part of the system (Hauck and Bremner, 1976).

The use of stable isotopes as a tracer requires: (1) that it be possible to change the natural abundance proportions of the different stable isotopes, and (2) that the chemical and biological processes to be studied, by the tracer techniques, are not able to differentiate between isotopes. In the case of N (separation and nonseparation of ^{15}N from ^{14}N) both the above requirements are fulfilled. Nitrogen labelling normally means an enrichment of the ^{15}N isotope. The degree of enrichment is usually expressed as the atom % excess over the natural atom percentage of the isotope. Since the natural abundance of ^{15}N is 0.366 atom %, excess of the enriched sample will be the atom percentage of ^{15}N minus 0.366. Different processes are used for the enrichment of stable isotopes: thermal diffusion, electromagnetic separation, electrolysis, fractional distillation, and various chemical exchange reactions (Bremner, 1965c).

An ^{15}N determination in tracer work involves the use of mass spectroscopy. Samples to be analyzed are converted to elemental N which is then injected into the mass spectrometer. The preparation of N samples for spectrometer analysis is normally performed in two steps: (1) conversion of the labelled N compounds to NH_4^+ and (2) conversion of NH_4^+ to N_2 by oxidation with alkaline sodium hypobromite in the absence of air. In the mass spectrometer, the N_2 molecules are ionized by exposure to a hot filament. The resultant molecular ions travel through a magnetic field where separation of the three molecular species of N ($^{14}N^{14}N$, $^{14}N^{15}N$, and $^{15}N^{15}N$) is made. Because the determination of isotopic composition is made on the basis of charged mass, care must be taken to minimize contamination by other charged particles having the same mass and charge as the N_2 molecules. For most mass spectrometers currently used in agricultural studies, comparatively large amounts of N (0.5-4 mg) are needed for precise and accurate analysis. Much smaller amounts of N (<10 μg) are used for N isotope ratio analysis by optical spectroscopy, but the precision obtained may be 10 to 100 times less than by mass spectroscopy (Hauck, 1982).

MATERIALS AND METHODS

1. Controlled Redox-pH Conditions

The soil used in this study was a Crowley silt loam (Typic albaqualf) collected from the Rice Experiment Station, Crowley, Louisiana, U.S.A. The soil has a C content of 0.60% (Nelson and Sommers, 1982) and a total N content of 649 mg N/kg with 30 mg N/kg in the NH_4^+ and NO_3^- form. Its pH was 5.6 in a 1:1 soil-water suspension (McLean, 1982).

The air-dried soil was ground and passed through a 40-mesh screen and mechanically mixed in a 4-liter Nalgene bottle for 2 days. Four hundred grams (on oven dry basis) of soil was mixed with 1600 ml deionized water in a two-liter flask (ratio 1:4, w/v). The study was carried out in microcosm system developed by Patrick et al. (1973) as described by Gambrell et al. (1980) with some modification by Hambrick et al. (1980). As shown in Figure 3, each flask was fitted with a combination pH electrode, 2 platinum electrodes, salt bridge serum cap, an air inlet, a gas outlet, N_2 inlet, thermometer and a stirring bar.

The pH electrode was connected to a potentiometer (Beckman Zeromatic IV pH meter). Redox potentials were monitored with platinum electrodes made by fusing an 18-gauge Pt wire with a lead glass tube. The two platinum electodes in each flask were connected, in order to obtain an average redox potential value, through a copper wire and alligator clips. The KCl -agar salt bridge coupled with a saturated calomel reference electrode and the platinum electodes completed an electrical circuit at the potentiometer (Cole Parmer pH/mV controller). The air inlet tube was connected in series through a water filled test tube and then to a needle value. The air needle valve was connected to an air pump which was switched on or off by a meter relay of the Cole Parmer pH/mv controller. The gas outlet was immersed in water in the gas trap bottle in order to prevent air from entering the flask. The N_2 was connected in series through a needle value, an O_2 removal trap (heated Cu metal) and a N_2 cylinder. A 10 cm PVC-teflon-coated magnetic

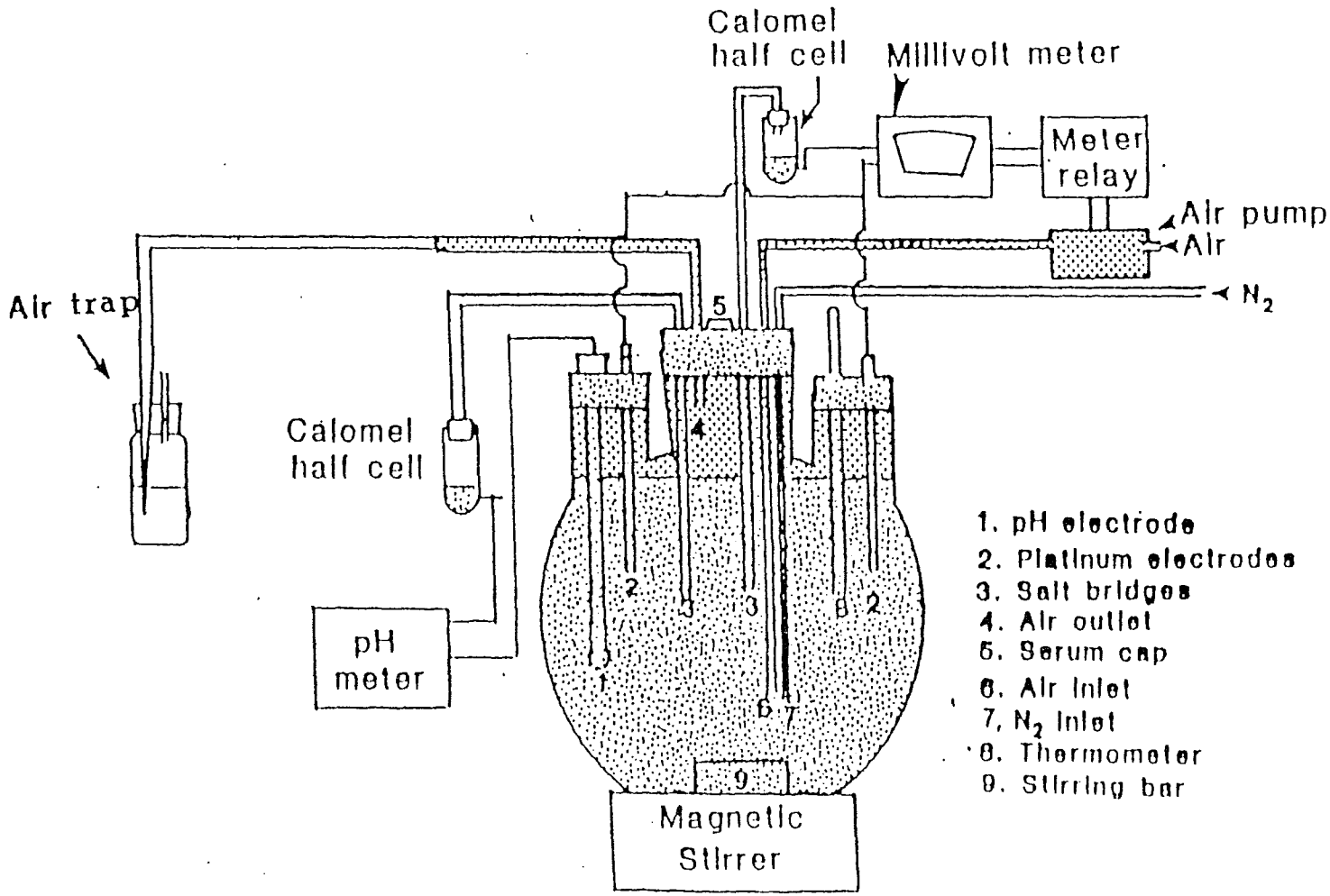

Figure 3. System used for incubating soil suspensions with labelled rice straw under controlled redox potential and pH conditions.

stirring bar was used to continuously stir the soil suspension. The temperature was controlled at 30°±1C. To prevent heat transfer from the stirrer to the suspension, insluation was inserted between the flask and the magnetic stirrer.

The redox potential and the pH of the system were monitored twice a day. Once the redox potential reached the desired value, the air needle valve was turned on to allow a slow flow of air. The preset Eh values were kept constant because the meter relay turned on or off the air pump depending on the prevailing conditions. There were four Eh treatments (-100, +100, +300, and +500 mV) and two pH treatments (pH 5 and pH 7). These pH and Eh treatments were factorially combined. Each treatment was replicated 3 times. During the study, suspension pH was adjusted to desired levels as necessary with manual additions of either 1.0 N HCl or 1.0 NaOH with a syringe and needle through the serum cap. After 2 weeks of preincubation, 0.5% of 60 mesh ^{15}N labelled rice straw with a C/N ratio of 52 was added to each flask. The redox potential and pH of the system containing the ^{15}N labelled straw were monitored, and aliquots were withdrawn weekly for determination of C_2H_2 reduction assay and inorganic and organic N throughout a 6-week period.

2. Acetylene Reduction Assay

A preliminary study was carried out for blank determinination, the influence of the period of incubation of soil-rice straw and C_2H_2 on C_2H_4 production. Samples were withdrawn at 4 hour intervals from the incubation flasks and C_2H_4 production was measured. Soil suspension aliquots were withdrawn with a syringe and needle inserted through the serum cap into the middle of the suspension that was flushed with N_2 gas. The N_2 gas prevented air penetration during sampling and a suck back of water from the gas outlet bottle. A 50-ml aliquot was transferred into a 250 ml-mason jar with sealed top and rubber septum. The vapor phase used in this assay was air, of which 10% was replaced by C_2H_2. After 24 hrs of incubation, a 15 ml gas sample was removed with a hypodermic

needle attached to a syringe and was stored in an evacuated tube. The samples were analyzed for C_2H_4 with a Perkin Elmer 3920 gas chromatography equipped with a flame ionization detector and a 1.83 m stainless steel column packed with Porapak N (80 to 100 mesh). The temperature settings were as follows: column temperature 50°C, interface temperature 150°C. Retention times were 32 and 72 seconds for C_2H_4 and C_2H_2, respectively. The soil samples were weighted after drying at 60°C.

3. Mineralization of Organic Nitrogen in Soil

3.1 Inorganic Nitrogen

Inorganic N (NH_4^+ and NO_3^-) analyses were performed on the same samples used for the C_2H_2 reduction assay. Each sample, containing approximately 22 g of dry soil, was extracted with 2N KCl (soil:salt ratio = 1:8). The soil solution was mechanically shaken for 1 hour and then vacuum filtered through Whatman no. 1 filter paper. Ammonium and NO_3^- were determined by stream distillation techniques as described by Keeney and Nelson (1982).

3.2 Organic Nitrogen

After the extraction of inorganic N, the soil samples were air dried. Two grams of soil from each sample were taken for the determination of organic N. To this soil weight 1.8 g catalyst mixture (100 g K_2SO_4 + 10 g $CuSO_4$ + 1 g Se) and 5 ml concentrated H_2SO_4 were added and digested on a digestion block. The digests were steam distilled with 10 M NaOH and N content determined as described by Bremner and Mulvaney (1982).

3.3 Nitrogen Isotope-Ratio Analysis

After titration, the distillates of both inorganic and organic N samples containing one mg N or more were acidified with 2 ml 0.08N H_2SO_4 to prevent volatilization of

NH_3. The collection jars were placed in the oven at 60-65°C and evaporated down to 3-5 ml and the contents were transfered to 20 ml scintillation vials with plastic cap liners to be evaporated to dryness, sealed and stored until isotope-ratio analysis were carried out on a Dupont Model 21-614 mass spectrometer. The method described by R. Hauck (1982) was followed. Ethylene production and atom % ^{15}N calculations are shown in Appendices A and B, respectively. Raw data are presented in Appendix C tables.

The data were analysed using the general linear model for repeated measures analysis of variance (SAS User's Guide: Statistics, 1985). To get rid of the three-way interaction (Eh*pH*Week) the data were transposed (SAS User's Guide: Basics, 1985). The Duncan's Multiple Range Test was used to compare the means and Pearson's Correlation was used to determine the relationships between the N forms. All analyses were carried out at 0.05 probability level.

RESULTS AND DISCUSSION

1. NITROGEN FIXATION

1.1 Blank Determination

To determine the ambient C_2H_4 concentration of water and C_2H_2, the use of blanks in the acetylene reduction technique was imperative. Acetylene was added to one set of samples and another set was without C_2H_2. After one minute equilibration, C_2H_4 was measured in each set. There was no detectable amount of C_2H_4 in either set. Fleet et al. (1975) reported that a failure to equilibrate the contaminating C_2H_4 results in great blank values which lower the values of total C_2H_4 evolved during incubations.

1.1 Influence of the Period of Incubation of the Soil and Rice Straw on Ethylene Production

The influence of the period of incubation of the soil and rice straw on the production of C_2H_4 is shown on Figure 4. A lag phase of about 12 hrs was observed. During that period no detectable amount of C_2H_4 was recorded. The microorganisms reducing C_2H_2 did not accumulate enough energy to carry out the reaction. However, after that period, the amount of C_2H_4 increased and reached its peak after about 24 hrs and then slowly decreased. This means that beyond 24 hrs of incubation excess C_2H_4 is produced and could be harmful to the microorganisms.

This lag phase arises from the dissolution of C_2H_4 formed during the reduction of C_2H_2 by nitrogenase in the soil solution and subsequent adsorption of the C_2H_4 by the soil particles. In soils, where solubility and adsorption of gases are expected to be important, the addition of ethylene in the gas mixture is recommended (Rinaudo et al. 1971).

1.3 Effect of Shaking on Gas Exchange

This test was undertaken to determine the period after which the equilibration of C_2H_4 between the aqueous and gas phases occurs. The results of Matsuguchi et al. (1979)

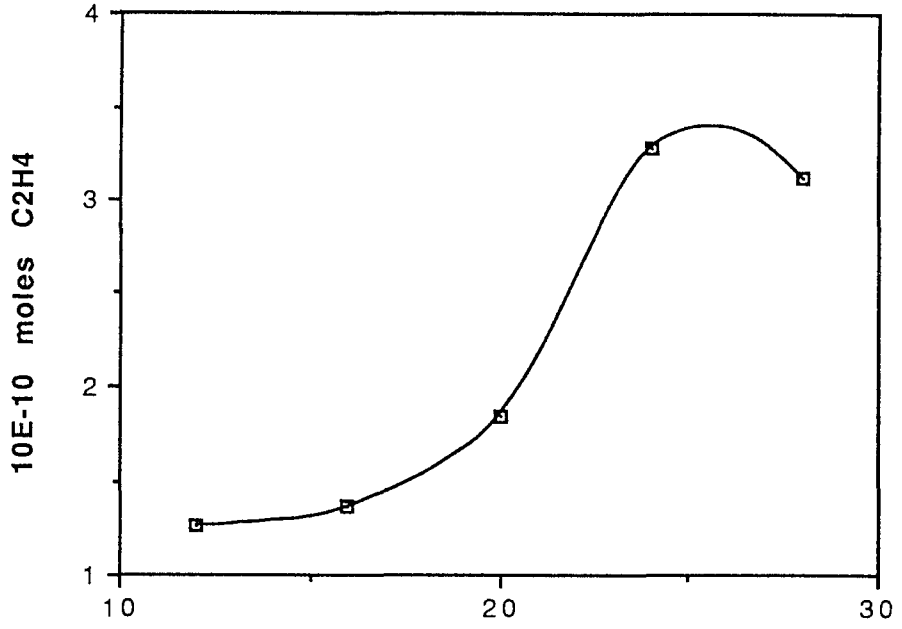

Figure 4: Determination of the lag period of C2H2 reduction in a Crowley silt loam amended with rice straw.

indicate that a definite procedure to achieve an adequate diffusion of C_2H_2 into the soil is crucial to the acetylene reduction assay for waterlogged soils. A set of mason jars containing the incubated suspension was handshaken for one minute; another set was shaken overnight on a rotary shaker. The handshaken set gave greater readings than the other set.

In this work, the magnitude of N_2 fixation is expressed in μmoles of C_2H_4 produced. Figure 5 shows the weekly production of C_2H_4 during the six week incubation period of the Crowley soil amended with labelled rice straw, at pH 7. The amount of C_2H_4 produced is approximately two times greater under anaerobic conditions (-100 and +100 mV) than under aerobic conditions (+300 and +500 mV). The weekly production of C_2H_4 at any redox potential level follows the same trend. It increases up to the second week then declines. The greatest production of C_2H_4 was recorded in the -100 mV treatment (the most reduced) at all weekly samplings. The second and third greatest production of C_2H_4 were obtained at +100 and +300 mV respectively. The lowest production of ethylene recorded at +500 mV (the most oxidized). The greatest weekly increase in production of C_2H_4 usually occurred in the second week of incubation (about 2.9 μmoles C_2H_4/kg/week).

The same general trend of C_2H_4 production noticed at pH 7 was observed at pH 5 (Figure 6). Here, also, the magnitude of the C_2H_4 production was about twice as great under anaerobic conditions as under aerobic conditions. However, compared to the production of C_2H_4 at pH 7, that at pH 5 showed a net decrease (7.9 μmoles C_2H_4/kg/week at -100 mV and pH 7 against 5.6 μmoles C_2H_4/kg/week at -100 mV and pH 5). There is a significant difference in the production of C_2H_4 at all redox treatments from the start of incubation of the rice straw throughout the fourth week. At the end of the experiment, the production of ethylene at -100 mV was significantly greater than those at all other redox treatments. The reverse was true for the production of C_2H_4 at -500 mV. The production of C_2H_4 was significantly greater at pH 7 than pH 5 at all weekly samplings.

Figures 7 and 8 depict the rates of C_2H_4 production and also show a significant redox potential-pH interaction. The general trend is the rate of C_2H_4 production increases with decrease in redox potential, but decreases with decrease in pH.

These results agree with the findings of several researchers that N_2 fixation is greater in anaerobic environment than in aerobic environment (Rice et al., 1967; Chang and Knowles, 1965; Yoshida and Acanjas, 1973; Yoneyama et al., 1977; Wada et al., 1978; Matsuguchi, 1979; Panichsakpatana et al., 1979). The greater N_2 fixation may be due to the prolific multiplication of *Clostridia* in presence of energy source (10^5 to 10^6 cells/g soil; Alexander, 1977). Rice et al. (1967) reported that *Clostridia* upon incubation in the laboratory increased 1000-fold in the anaerobic soil and 100-fold in the field-capacity soil. Unlike the *Clostridia*, *Azotobacter*, which are the most encountered aerobic N fixers are not found in dense colonies (10^3 to 10^4 cells/g soil). Moreover, they possess the greatest respiration rate. Since N2 fixation and respiration are two competitive reactions, the N_2 fixation efficiency of *Azotobacter* is low. It is also known that O_2 inactivates the Fe-protein, component of nitrogenase (Mulder and Brotonegoro, 1974). Yoshida and Ancajas (1973) speculated that the movement of carbohydrate to the rhizosphere or the accumulation of carbohydrates by aerobic microbial decomposition to CO_2 and water may be limited under anaerobic conditions. They indicated that under anaerobic conditions the carbohydrate converted to organic acids are good energy sources for N_2-fixing organisms. Watanabe (1978) suggested that the slow transfer and rapid consumption of O_2 in flooded soil is probably one of the reasons for the low sensitivity of N_2 fixation under aerobic conditions. Yoneyama and Yoshida (1977) stated that the peak of N_2 fixation coincides closely with the time when the N_2 immobilization by rice straw occurs.

Our results confirmed the previous findings, that N_2 fixation is greater at neutral pH than at acidic pH (Alexander, 1977; Yoneyama et al., 1977; Matsuguchi, 1979; Panichsakpatana et al., 1979; Van Verkum and Sloger, 1982). Here again, this can be explained by the activity of the microorganisms involved. *Azotobacter* is known to be very

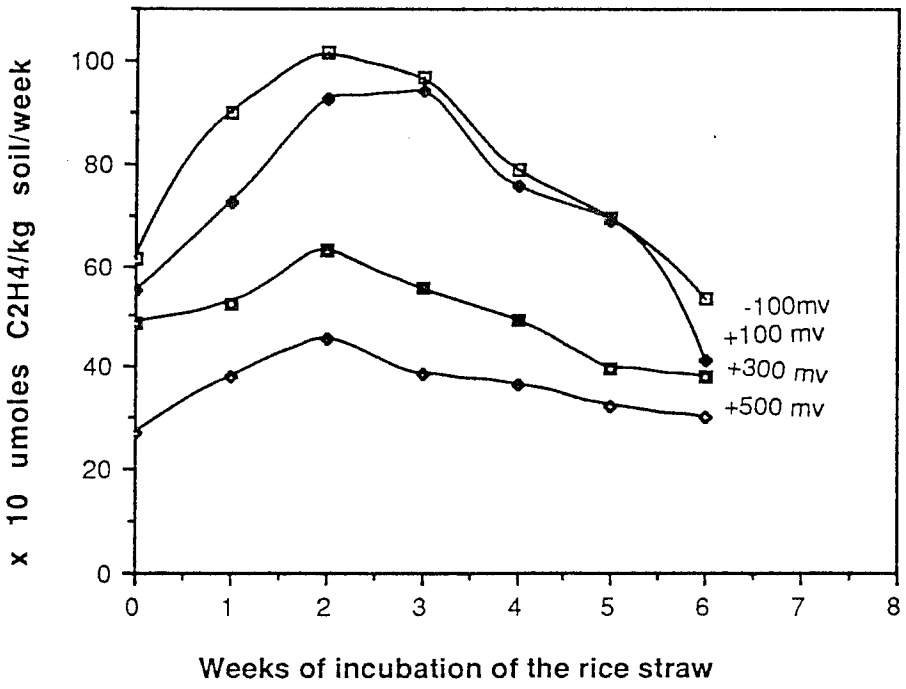

Figure 5: Changes in C2H4 production in a Crowley silt loam at pH 7 and varied redox potentials

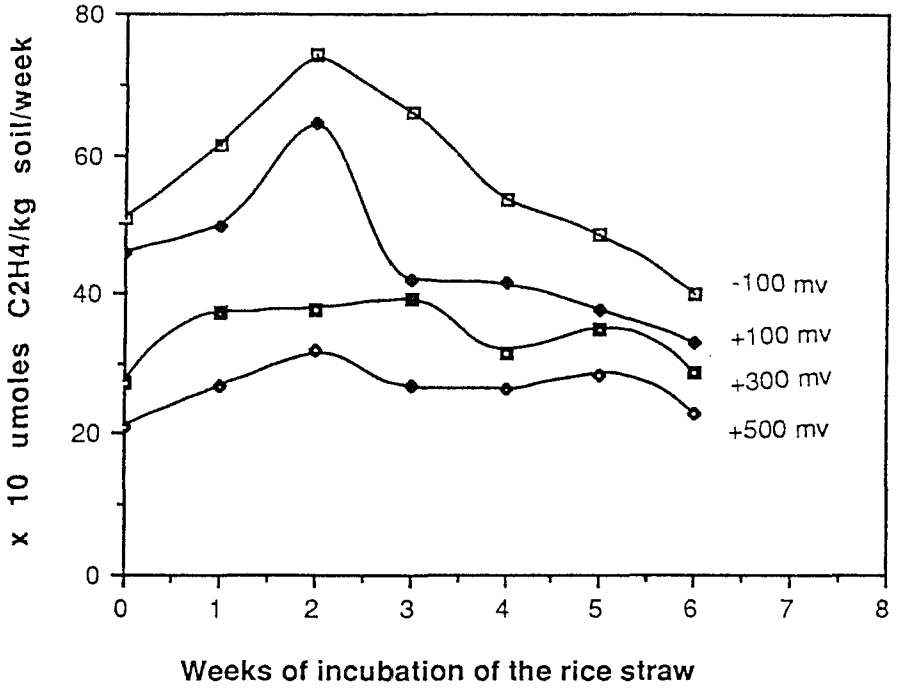

Figure 6: Changes in C2H4 production in a Crowley silt loam at pH 5 and varied redox potentials.

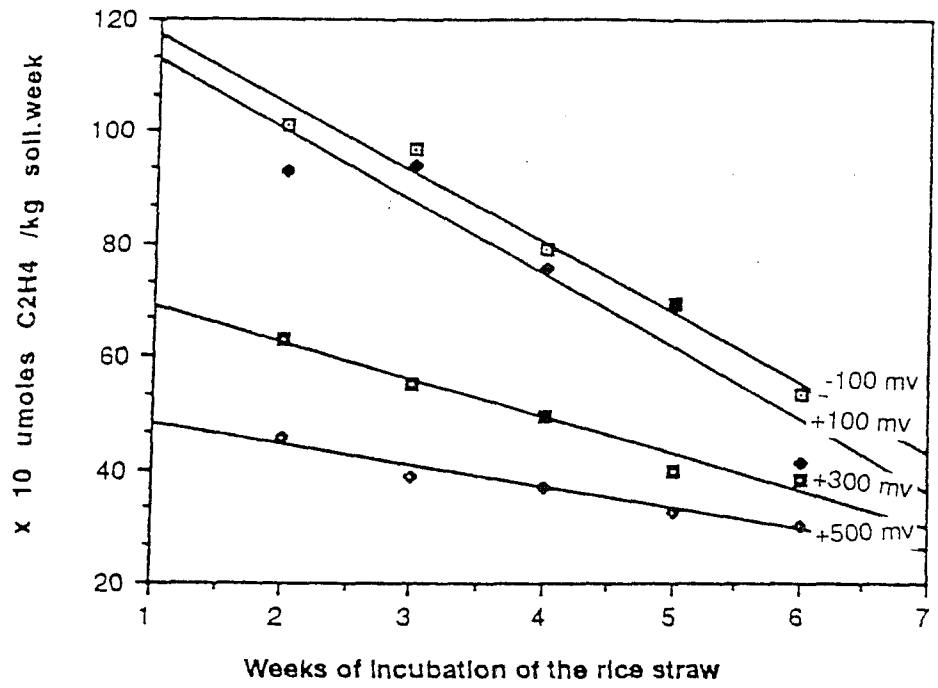

Figure 7: Rate of change in C2H4 production in a Crowley silt loam at pH 7 and varied redox potentials.

-100 mV	y = 129.20 - 12.335 X	R = 0.976
+100 mV	y = 125.58 - 12.766 X	R = 0.886
+300 mV	y = 75.178 - 6.499 X	R = 0.970
+500 mV	y = 51.565 - 3.715 X	R = 0.956

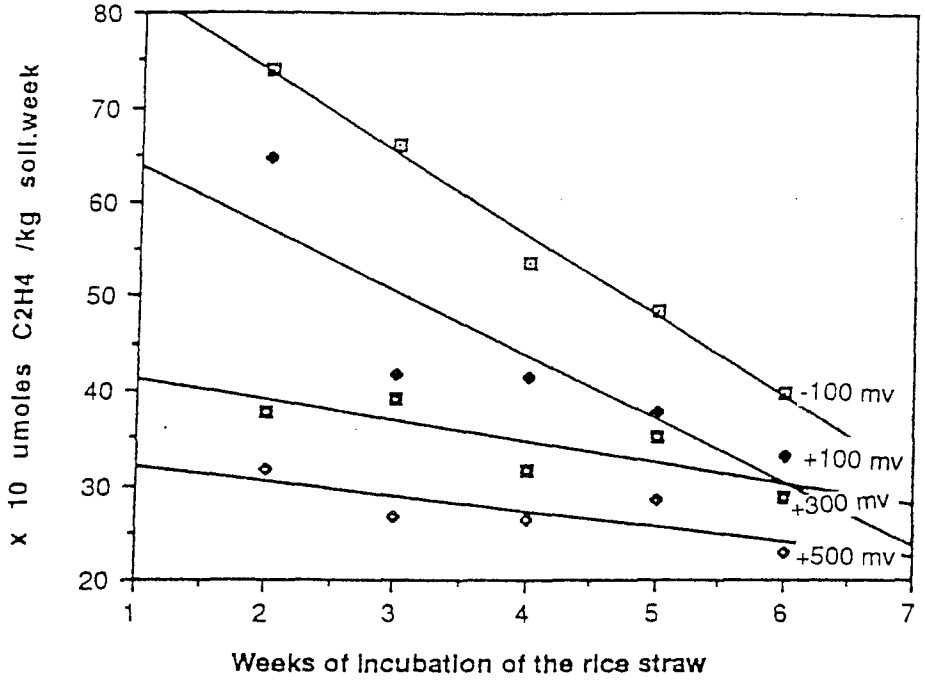

Figure 8: Rate of change in C2H4 production in a Crowley silt loam at pH 5 and varied redox potentials.

-100 mV	y = 90.766 - 8.597 X	R = 0.987
+100 mV	y = 70.576 - 6.723 X	R = 0.761
+300 mV	y = 43.424 - 2.235 X	R = 0.666
+500 mV	y = 33.508 - 1.578 X	R = 0.593

sensitive to acidity, unlike *Clostridium*, which has a larger range of pH tolerance. Yoneyama and Yoshida (1977) suggested that a large amount of organic acid produced as a result of the decomposition of rice straw might depress the microbial activities. Matsuguchi (1979) prescribed liming for acid rice fields of Thailand to enhance their N_2 fixation.

2. MINERALIZATION OF NITROGEN

Figures 9 and 10 summarize the changes in the organic N (labelled and non-labelled at pH 7 and 5, respectively). These changes scrupulously followed the redox potential levels with significantly greater organic N contents at low redox potential levels than at great redox potential levels. The decrease in total N over time reflects the mineralization at the various redox potential and pH values investigated. Figures 11 and 12 show the rates of mineralization at pH 7 and pH 5, respectively. The rate of mineralization generally decreases with increase in redox potential at both pH values.

Figures 13 and 14 illustrate the changes in extractable NH_4^+ contents at pH 7 and pH 5, respectively. The extractable NH_4^+ contents follow almost the same pattern regardless of pH level. However, there is a tremendous difference between the release of extractable NH_4^+ at -100 mV and those at the other redox potential levels, and this is noticeable at both pH levels. Significantly greater amounts (0.05 level) of NH_4^+ were released at pH 5 than pH 7. By the end of the second week at -100 mV and pH 5, as much NH_4^+-N (21 mg N/kg) was released as at the end of the third week at pH 7 and the same redox potential level (20 mg N/kg). The amount of NH_4^+-N released at the end of the incubation period of the rice straw was 55 mg N/kg soil at -100 mV and pH 7 against 70 mg N/kg soil at the same redox potential at pH 5. The greatest weekly release of NH_4^+-N was recorded in the fourth week (17 mg N/kg soil). The amount of organic N mineralized decreased in the fifth week, but exploded again in the last week of incubation. At the other redox levels the rate of mineralization was comparatively very low. However, it was found in this experiment

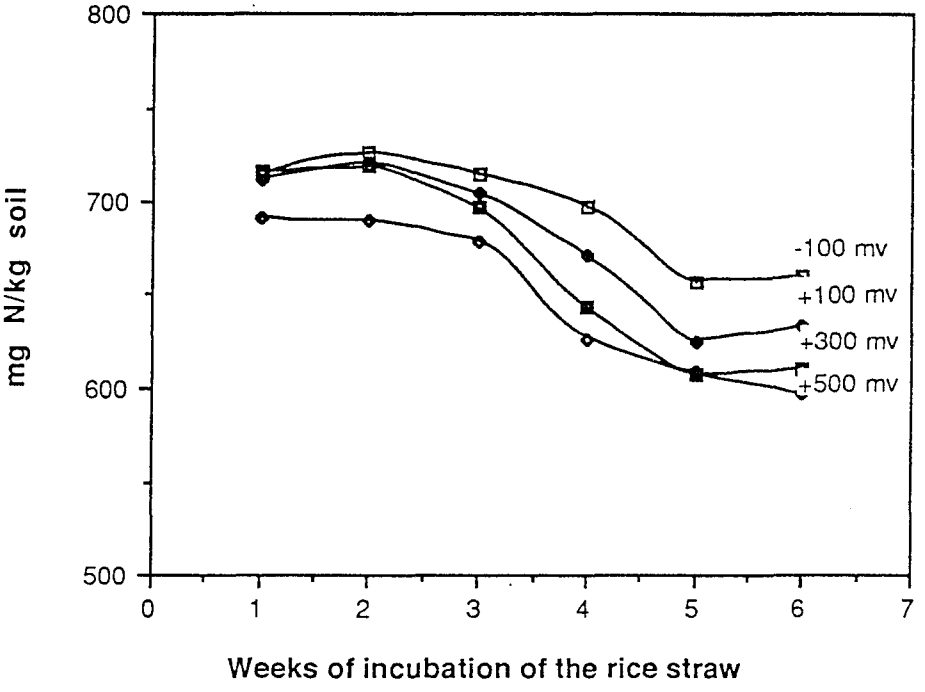

Figure 9: Changes in the organic N in a Crowley silt loam at pH 7 and varied redox potentials.

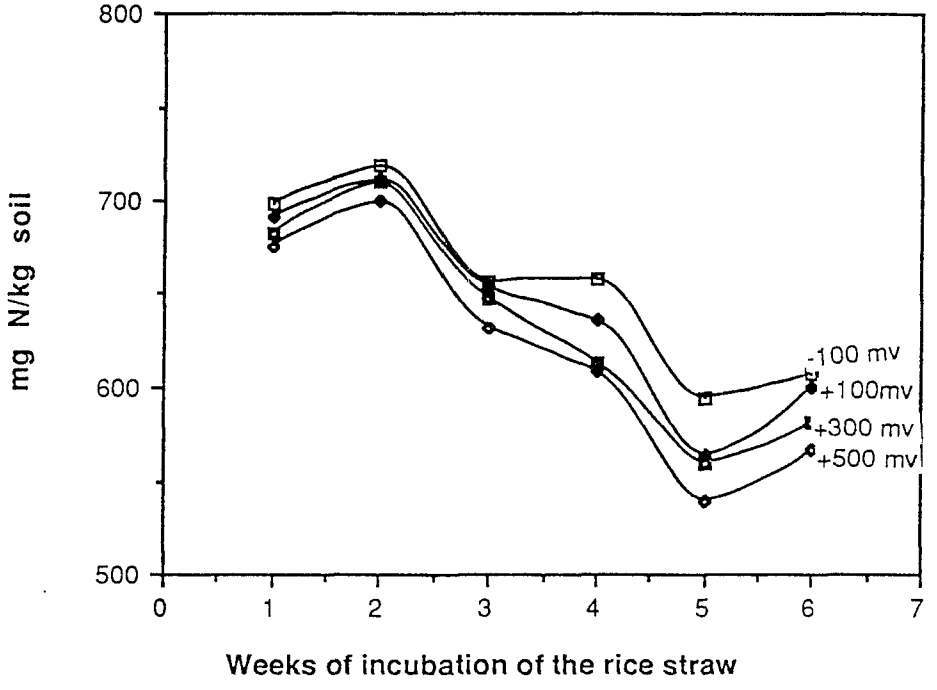

Figure 10: Changes in organic N in a Crowley silt loam at pH 5 and varied redox potentials.

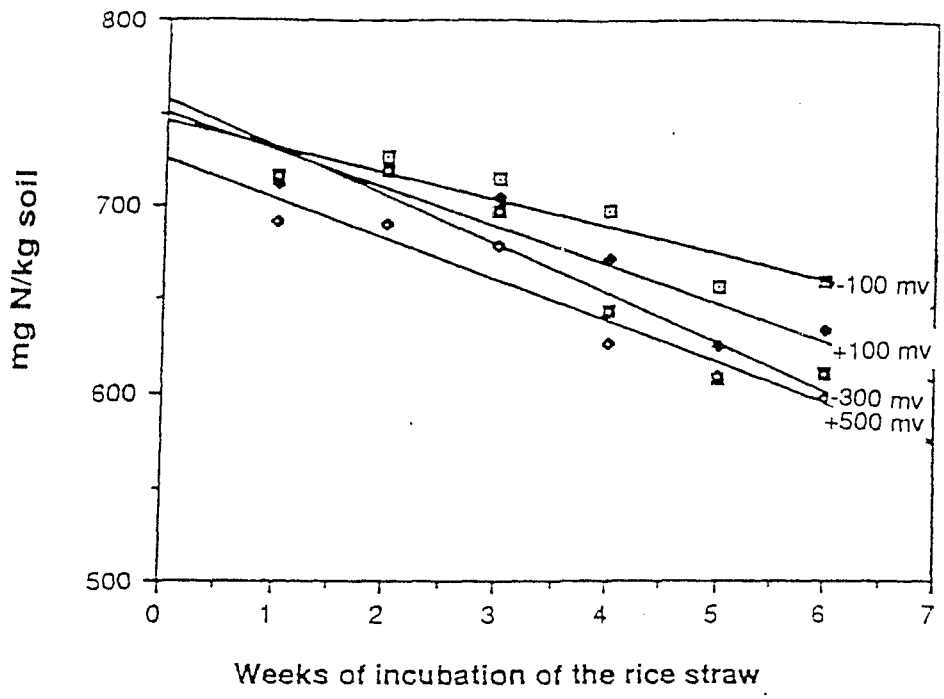

Figure 11: Rate of change in the organic N in a Crowley silt loam at pH 7 and varied redox potentials.

-100 mV	y = 744.44 - 14.231 X	R = 0.801
+100 mV	y = 748.60 - 20.310 X	R = 0.855
+300 mV	y = 756.62 - 26.161 X	R = 0.903
+500 mV	y = 725.09 - 21.899 X	R = 0.915

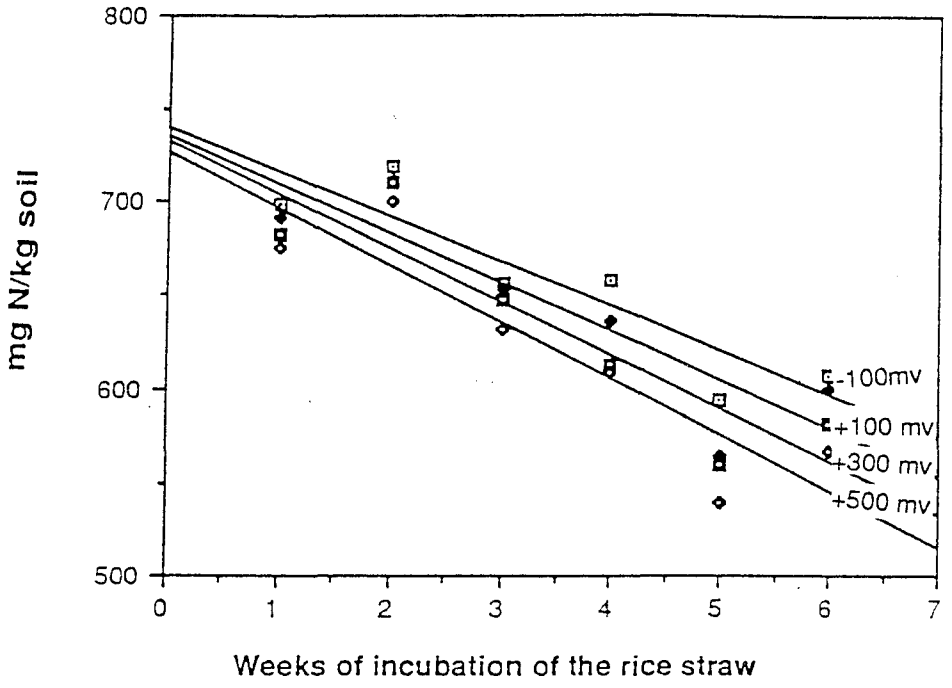

Figure 12: Rate of change in the organic N in a Crowley silt loam at pH 5 and varied redox potentials.

-100 mV	y = 738.88 - 23.749 X	R = 0.824
+100 mV	y = 734.58 - 26.189 X	R = 0.780
+300 mV	y = 732.28 - 28.568 X	R = 0.827
+500 mV	y = 726.01 - 30.167 X	R = 0.829

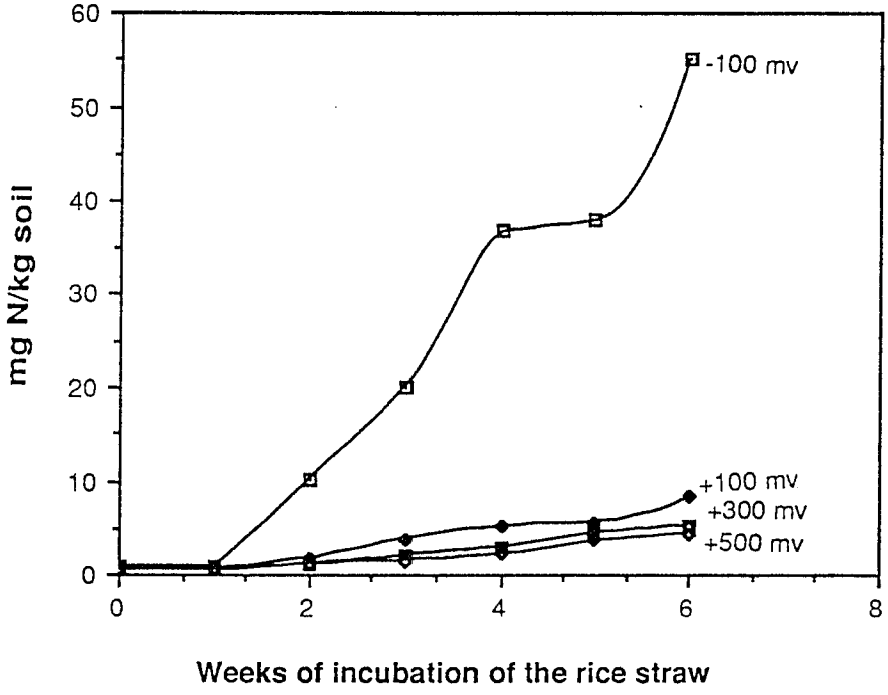

Figure 13: Changes in extractable NH_4^+-N in Crowley silt loam at pH 7 and varied rodox potentials.

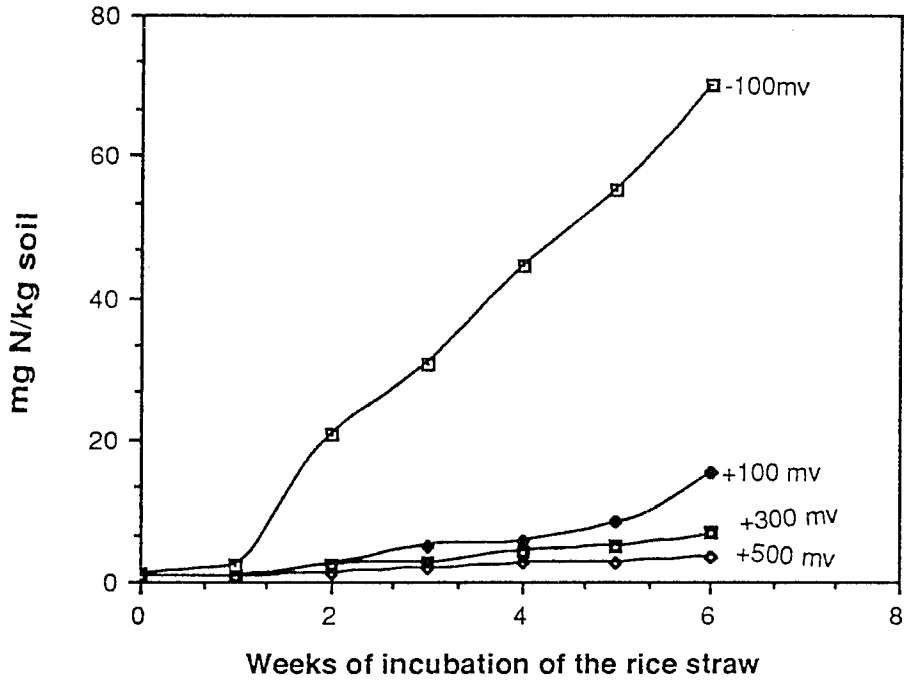

Figure 14: Changes in extractable NH_4^+-N in Crowley silt loam at pH 5 and varied rodox potentials.

that the mineralization rate decreased with increase in redox potential to pH 7 as well as pH 5 (Figures 15 and 16).

In any soil system, there is a continuous interchange between the organic and inorganic N pools. Broadbent (1978) indicated two reasons for the low N requirements of the anaerobic microorganisms. They are inherently less efficient than aerobic microorganisms in providing energy for the build-up of new cells. Many of the general-purpose microorganisms like the actynomycetes which assimilate 15-30 % of substrate C to microbial cell, and fungi, which convert up to 40 % of substrate C are virtually absent under anaerobic conditions. Thus, the organic N is better preserved under anaerobic than aerobic conditions, and the greater immobilization rates at lower redox potential are to be expected. In fact, the decrease in mineralization rates with increase in redox potential is in agreement with the results of Waring and Bremner (1964). Williams et al. (1968) reported 0.5% N required for the decomposition of rice straw against 1.5% N under anaerobic and aerobic conditions respectively.

3. NITRIFICATION

The dynamics of NO_3^--N during the six weeks of rice straw incubation is depicted in Figures 17-20. Starting from the third week of incubation differences in nitrate production were observed at different redox and pH levels. At -100 and +100 mV there was no detectable amounts of NO_3^--N at either pH. The greatest amounts of NO_3^--N were recorded at pH 7. More NO_3^--N was produced at 500 mV than 300 mV at any given pH. The nitrification rate is slightly greater at 500 mV than 300 mV. It is significantly greater at pH 7 than pH 5. There is a significant redox pH interaction (Figures 19 and 20).

Nitrification is the biological oxidation of NH_4^+-N to NO_3^--N. Thus, the reaction is inhibited in O_2-deficient environments. Theoretically, O_2 becomes undetectable at redox potential of 350 mV. This explains why more nitrate was recorded at 500 mV than 300 mV. However, nitrification occurs in wetland rice soil, in the flood water and oxidized soil

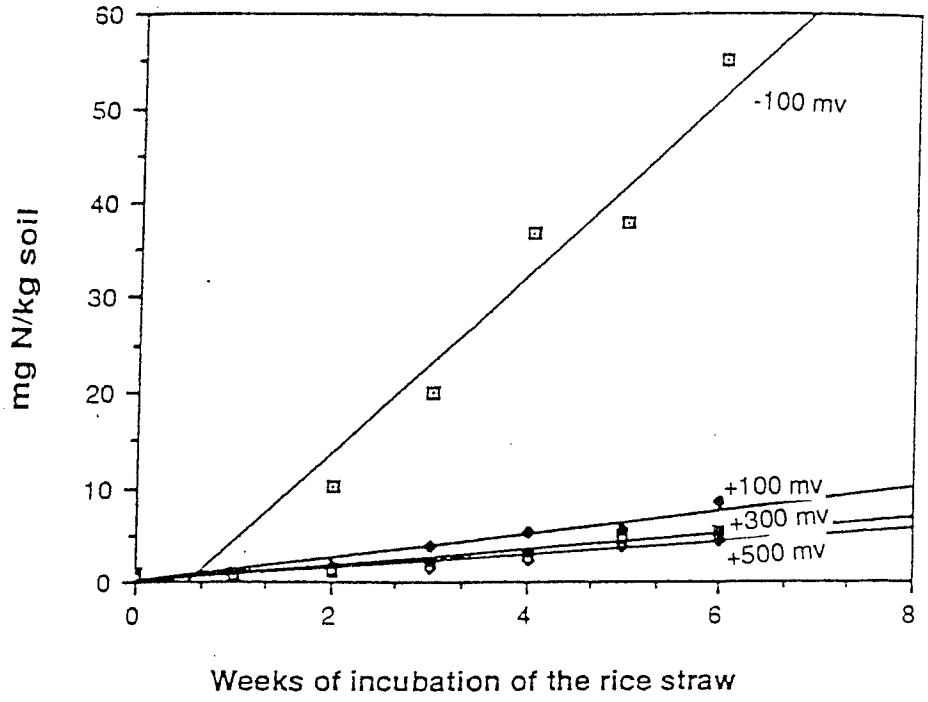

Figure 15: Rate of change in the extractable NH_4^+-N in Crowley silt loam at pH 7 and varied rodox potentials.

-100 mV	$y = -5.1503 + 9.3953\ X$	R = 0.953
+100 mV	$y = -7.1928\ (10^{-2}) - 1.2731\ X$	R = 0.947
+300 mV	$y = -2.1964\ (10^{-2}) + 0.8343\ X$	R = 0.934
+500 mV	$y = 3.3393\ (10^{-2}) + 0.66325\ X$	R = 0.914

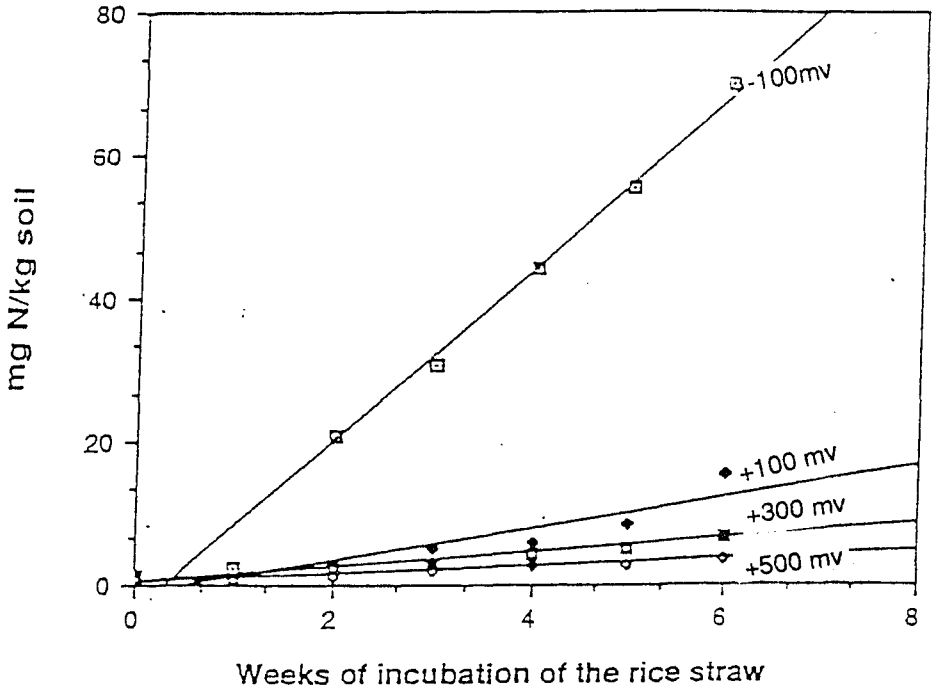

Figure 16: Rate of change in the extractable NH_4^+-N in Crowley silt loam at pH 5 and varied rodox potentials.

-100 mV	y = -4.0242 + 12.022 X	R = 0.983
+100 mV	y = -1.129 + 2.213 X	R = 0.859
+300 mV	y = 0.233 + 1.008 X	R = 0.962
+500 mV	y = 0.424 + 0.499 X	R = 0.963

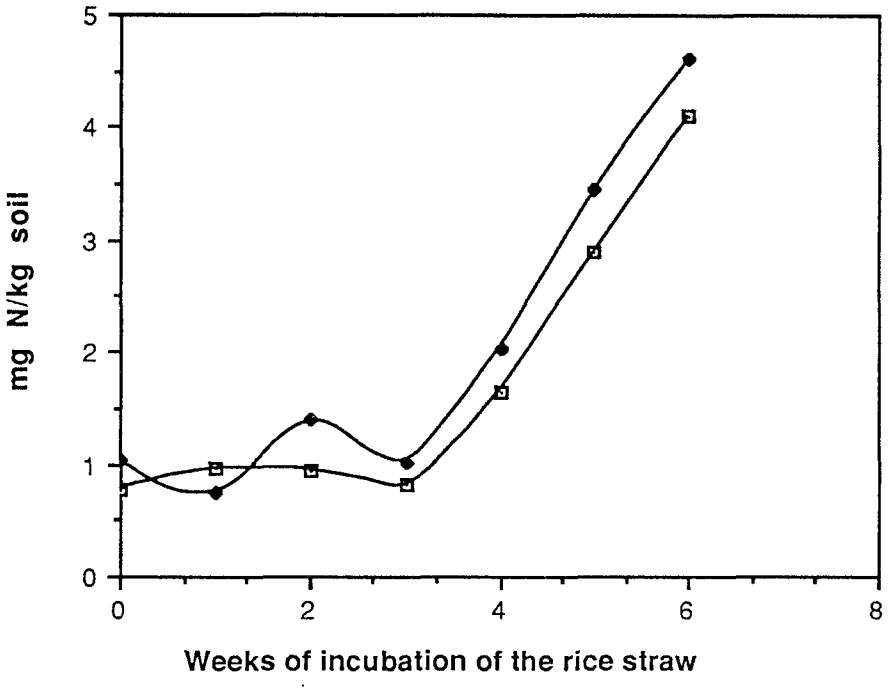

Figure 17: Changes in NO_3^--N in a Crowley silt loam at pH 7 and varied redox potentials.

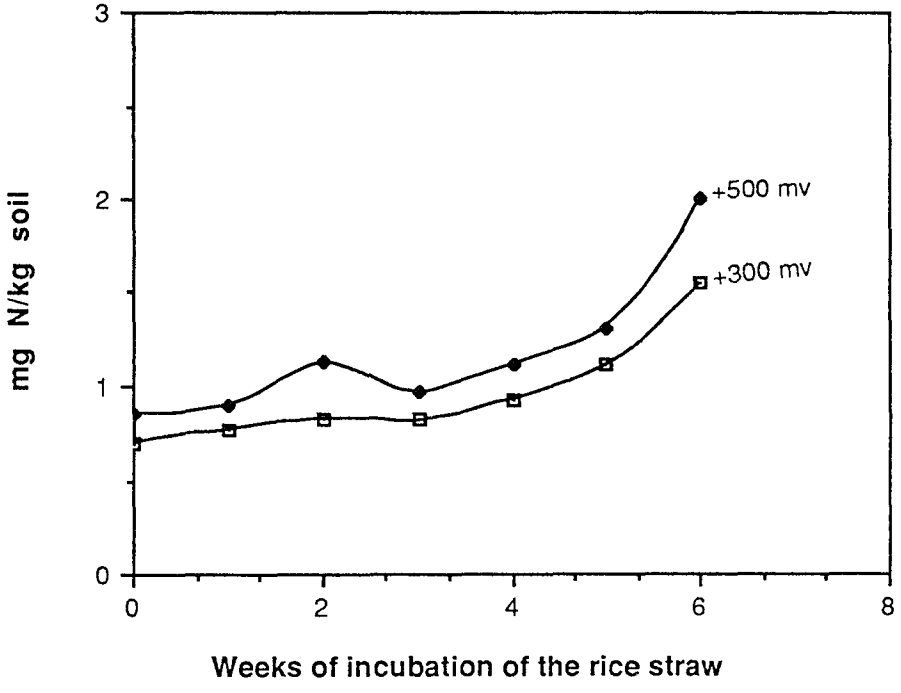

Figure 18: Changes in NO_3^--N in a Crowley silt loam at pH 5 and varied redox potentials.

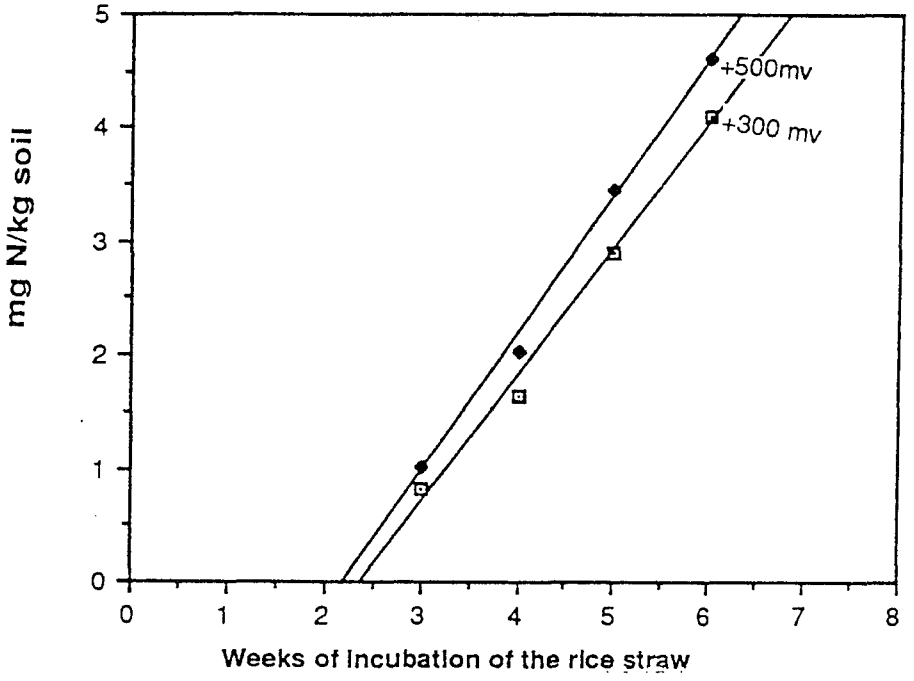

Figure 19: Rate of change in NO_3^--N in a Crowley silt loam at pH 7 and various redox potentials.

+300 mV	y = -2.652 + 1.116 X	R = 0.993
+500 mV	y = -2.701 + 1.218 X	R = 0.997

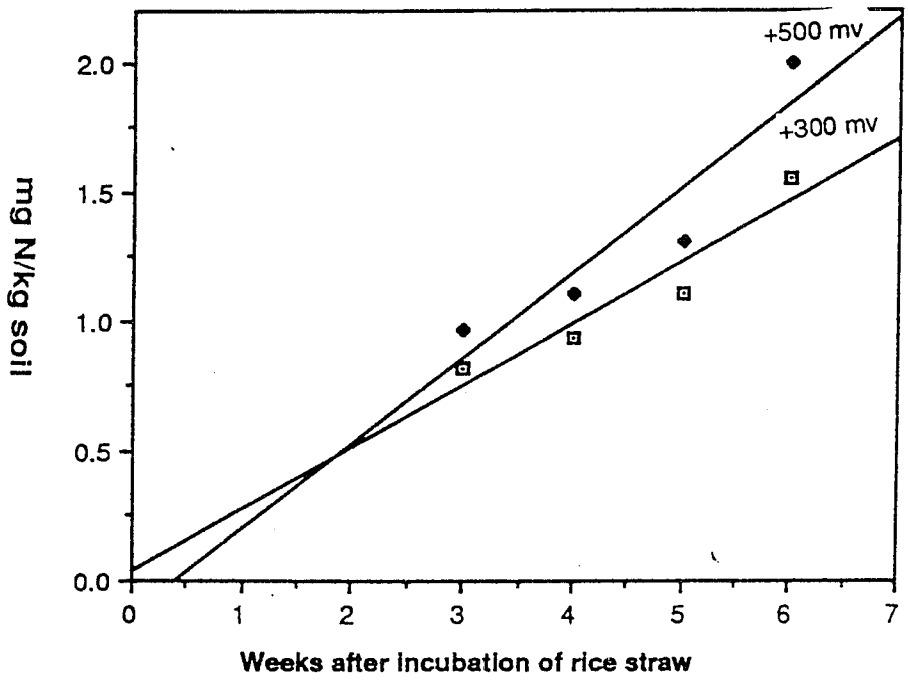

Figure 20: Rate of change in NO_3^--N in a Crowley silt loam at pH 5 and various redox potentials.

+300 mV	$y = 3.600 \times 10^{-2} + 0.237 X$	R = 0.906
+500 mV	$y = -0.140 + 0.330 X$	R = 0.863

layer. Nitrification is a two-step reaction. First, NH_4^+ is oxidized to NO_2^- which subsequently is oxidized to NO_3^-. The first reaction is carried out by *Nitrosomonas* or related genera and the second reaction by *Nitrobacter* or related genera. In acid environments, nitrification is suppressed. Low pH affects not only the transformation of NH_4^+ to NO_3^- but also the population of the nitrifiers. The rate of nitrification falls off markedly below pH 6.0 and becomes negligible below 5.0 (Dancer et al., 1973). The optimum pH for nitrification is in the neighborhood of 7 (Alexander, 1977).

4. LABELLED NITROGEN FRACTIONS

4.1 Labelled Organic Nitrogen

The changes in labelled organic N are illustrated in Figures 21-24. The general pattern in the dynamics of the labelled organic N is similar to that of the total organic N. The decrease in the labelled organic N fraction over time reflects net mineralization of the added labelled rice straw to the soil. This is in contrast to the total organic N decreases observed in Figures 9 and 10, which reflect net mineralization from both the soil and labelled rice straw. In addition, immobilization, remineralization and denitrification may also be occurring to varying degrees depending upon pH and soil redox potential constraints. An overwhelming part of the added labelled N (77 to 96%) remained in the organic form (Table 1). Except in the fourth week, there is no significant difference between the redox treatments as far as the amounts of labelled organic N is concerned. Asami (1971) reported that hydrolyzable NH_4^+, α-amino-N, and nonhydrolyzable fractions are the major fractions of the labelled organic N. Tusneem and Patrick (1971) reported 41% of immobilized N in the alpha-amino fractions in a flooded soil incubated with rice straw compared with 14% in all other fractions combined, with only negligible amounts in the nonhydrolyzable fraction. Regardless of the source of N in soil (plant residues, microbial cell, or inorganic fertilizers), Broadbent (1979) indicated that after the process of humification has taken place a major portion of the N is locked up into great

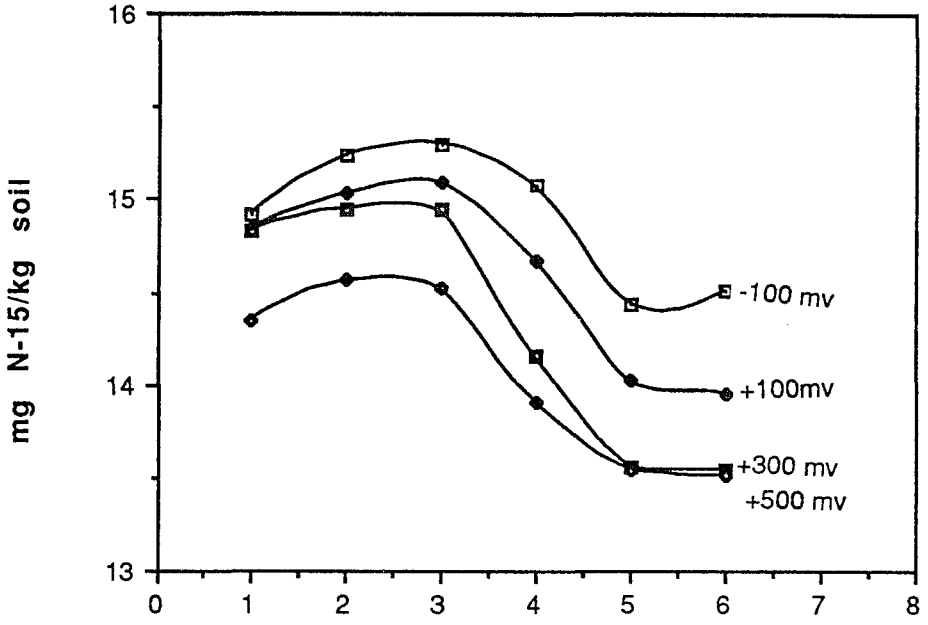

Figure 21:Changes in labelled organic N in a Crowley silt loam at pH 7 and varied redox potentials.

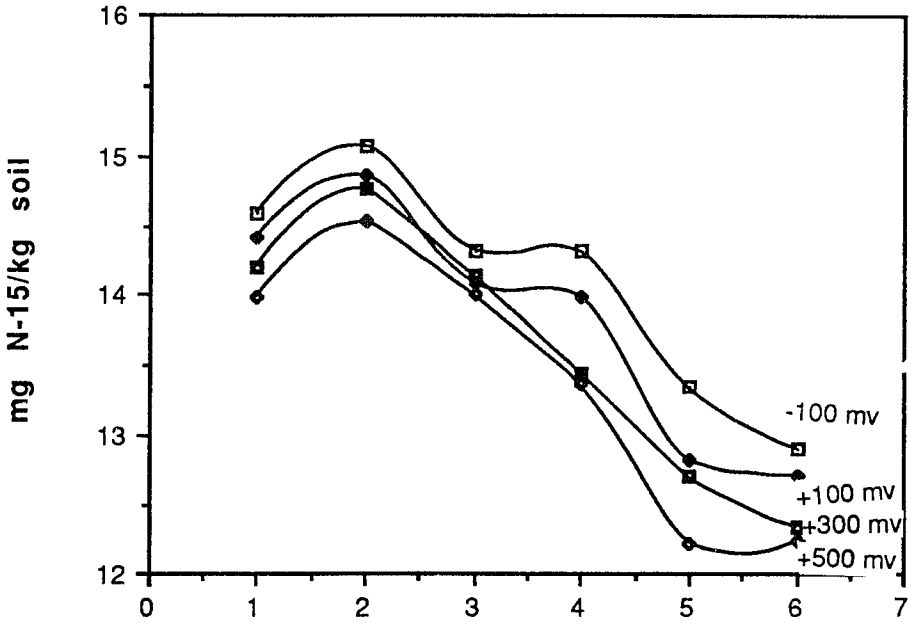

Figure 22: Changes in labelled organic N in Crowley silt loam at pH 5 and varied redox potentials.

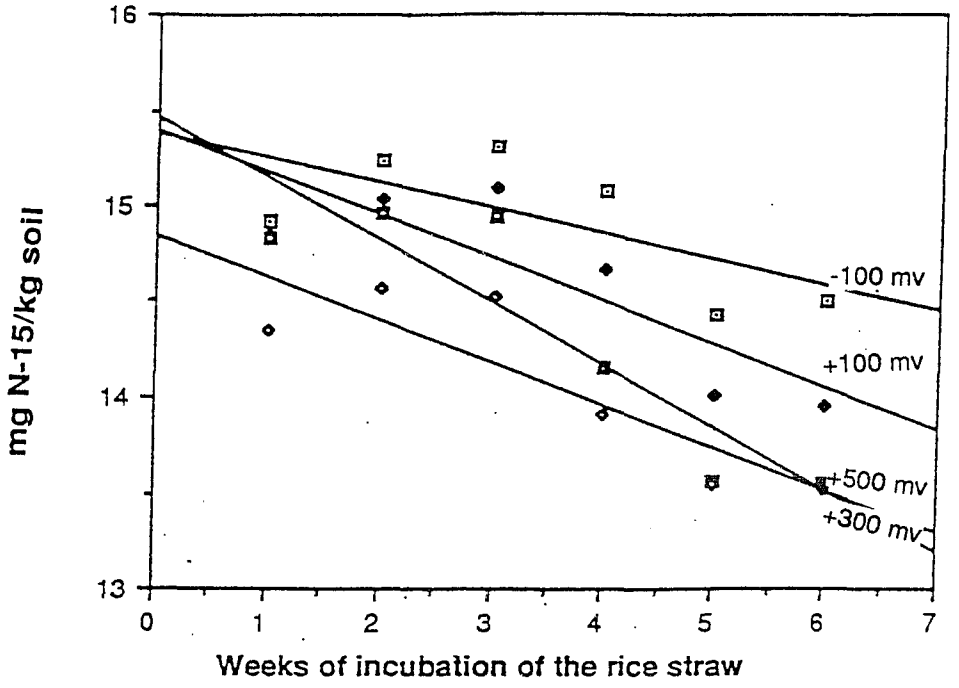

Figure 23: Rate of change in labelled organic N in a Crowley silt loam at pH 7 and varied redox potentias.

-100 mV	y = 15.377 - 0.133 X	R = 0.714
+100 mV	y = 15.397 - 0.224 X	R = 0.467
+300 mV	y = 15.457 - 0.324 X	R = 0.823
+500 mV	y = 14.847 - 0.223 X	R = 0.774

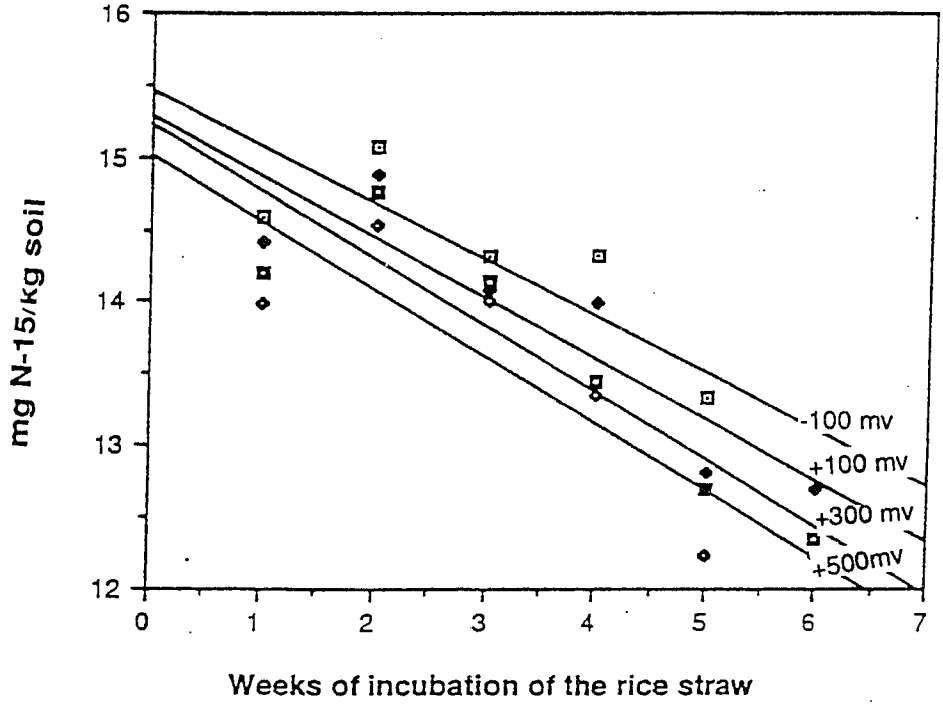

Figure 24 : Rate of change in labelled organic N in a Crowley silt loam at pH 5 and varied redox potentials.

-100 mV	y = 15.459 - 0.391 X	R = 0.805
+100 mV	y = 15.293 - 0.423 X	R = 0.828
+300 mV	y = 15.226 - 0.465 X	R = 0.848
+500 mV	y = 15.021 - 0.465 X	R = 0.806

Table 1. Labelled Organic Nitrogen
(Expressed as % of added labelled-N)

pH	mV	Weeks 0	1	2	3	4	5	6
7	-100	88.50	93.25	95.19	95.63	94.19	90.25	90.69
	+100	87.50	92.75	93.94	94.31	91.69	87.63	87.25
	+300	85.94	92.69	93.44	93.38	88.50	84.75	84.69
	+500	85.38	89.69	91.00	91.13	86.94	84.69	84.50
5	-100	87.31	91.19	94.25	89.50	89.50	83.36	80.63
	+100	86.25	90.06	92.94	88.06	87.44	80.13	79.38
	+300	84.63	88.81	92.31	88.38	84.00	79.38	77.44
	+500	84.56	87.44	90.81	87.50	83.50	76.44	76.50

molecular weight compounds which are resistant to decomposition. Usually more immobilization of inorganic N to organic N occurs under reduced conditions as compared to oxidized soil conditions. The lack of significant effect of redox potential is probably due to the relatively short period of incubation. The greater mineralization of labelled organic N at pH 7 is probably due to more microbial activity at this pH. Similar results were reported by Reddy and Patrick (1979) in an experiment where the Crowley soil was incubated with labelled $(NH_4)_2SO_4$

4.2 Labelled Extractable Ammonium

The changes in the amounts of labelled extractable NH_4^+ released are shown in Figures 25 and 28. Detectable amounts of labelled extractable NH_4^+ were recorded only at -100 mV and +100 mV at both pH 7 and pH 5, and this occurs in the fifth week of incubation. However, at that period at -100 mV, the amount of labelled organic N mineralized at pH 5 was about twice that mineralized at the same redox potential at pH 7 (1.66 mg ^{15}N/kg soil against 0.79 mg ^{15}N/kg soil). A sharp decline in the mineralization of this labelled N fraction was observed in the sixth week. The labelled extractable ammonium released was significantly greater than at pH 7 the last three weeks of incubation. Its amounts released are expressed in % of added labelled N in Table 2. The labelled ammonium released ranged from 2.63% to 10.38% of the total labelled N. This is particularly true at lower redox potential and neutral pH conditions. Figures 25 and 26 show lower rate: 9.3×10^{-2} $\mu g^{15}N$/kg was found at -100 mV and pH 7 compared to 7.0×10^{-2} $\mu g^{15}N$/kg. However at pH 5 the mineralization rate of the labelled organic N was greater at -100 mV (0.21 mg ^{15}N/kg) than at +100 mV (-0.14 mg ^{15}N/kg). A significant redox potential -pH interaction was found in the amounts of labelled NH_4^+ released.

These results are in agreement with the fundings of several researchers. Tusneem and Patrick (1971) indicated that remineralization of immobilized N is a slow process under anaerobic conditions. Kai and Kawaguchi (1977) showed that a large portion of the

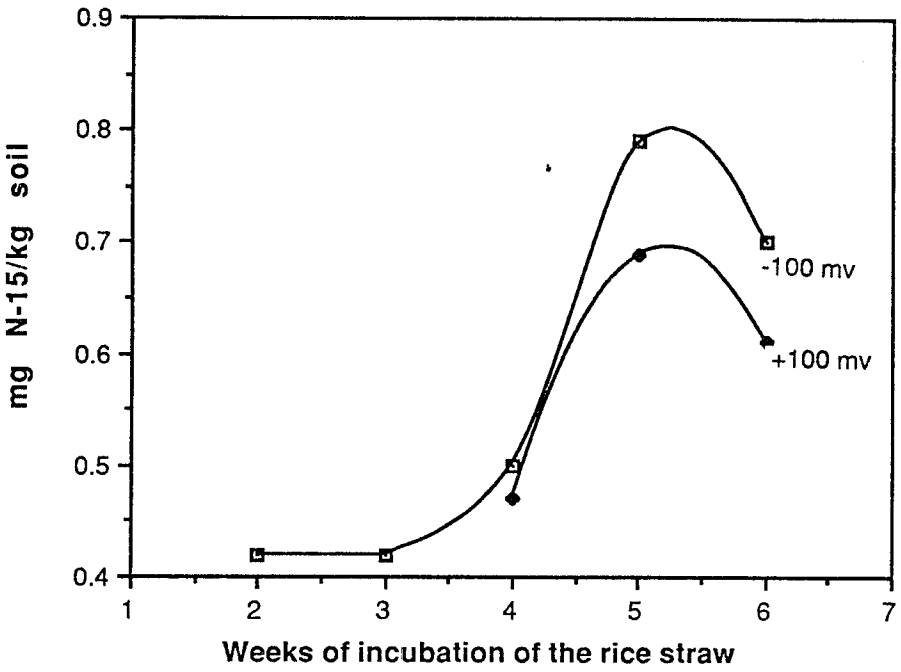

Figure 25: Changes in labelled NH_4^+-N in a Crowley silt loam at pH 7 and varied redox potentials.

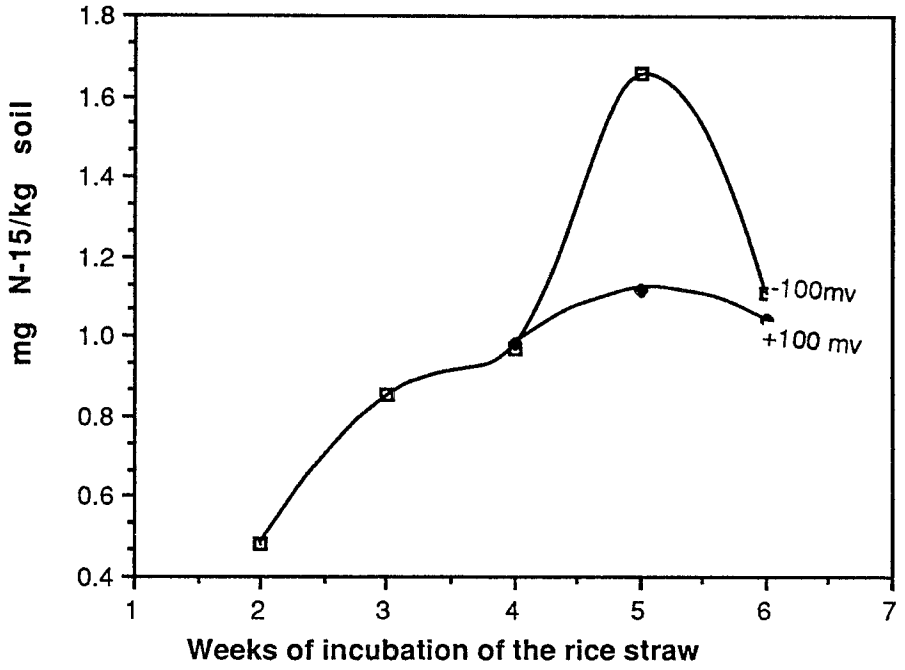

Figure 26: Changes in labelled NH_4^+-N in a Crowley silt loam at pH 5 and varied redox potentials.

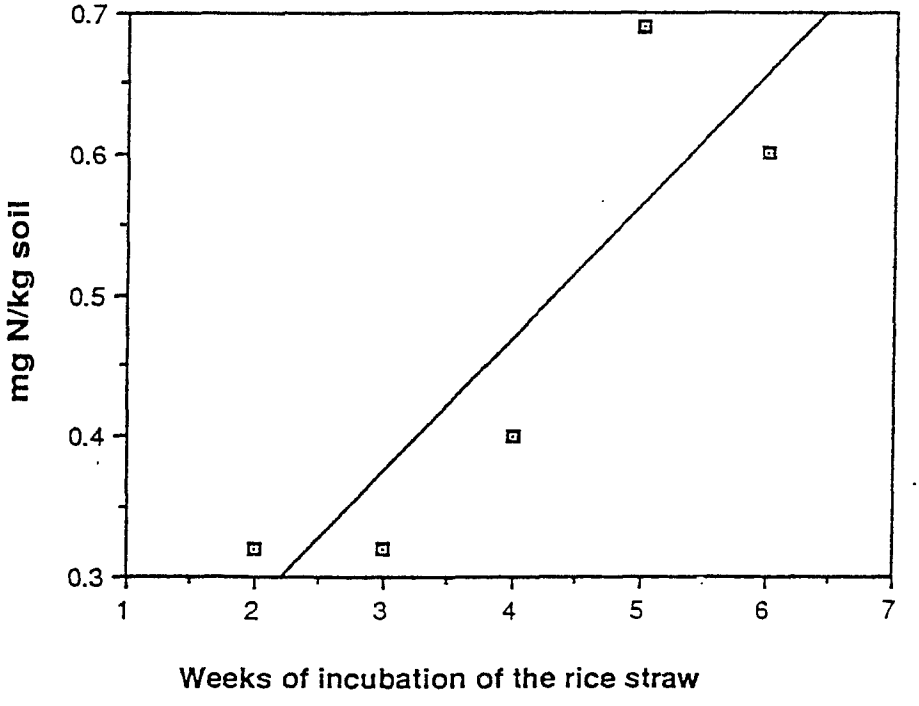

Figure 27: Rate of change in labelled NH_4^+-N in a Crowley silt loam at pH 7 and -100 mv.

100 mV at pH 7 $y = 0.194 + 9.300 \times 10^{-2} X$ $R = 0.751$

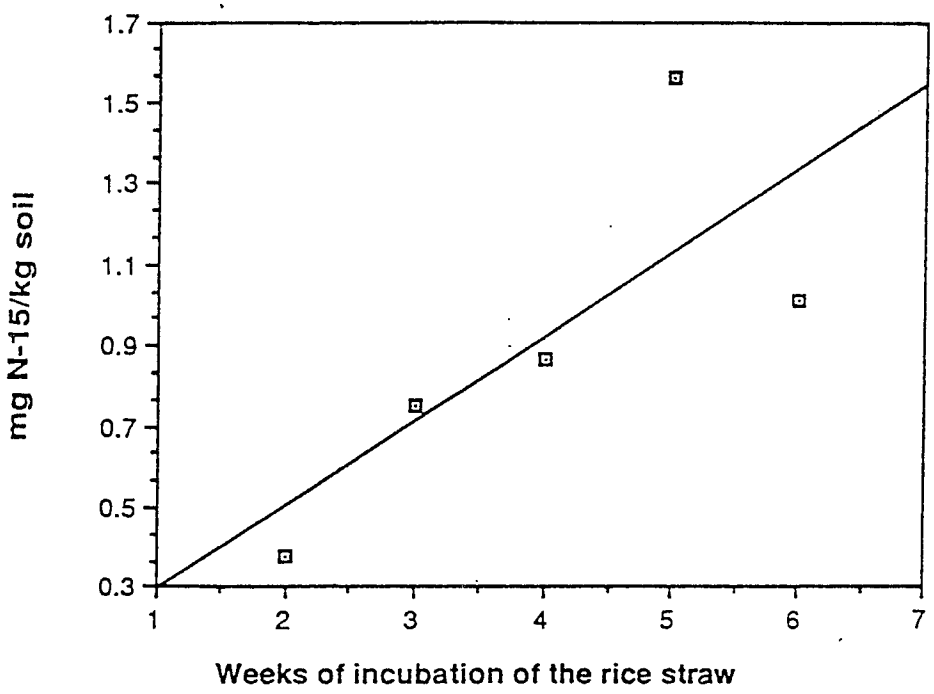

Figure 28: Rate of change in labelled NH_4^+-N in a Crowley silt loam at pH 5 and -100 mv.

100 mV at pH 5 $y = 0.186 + 0.207 X$ $R = 0.579$

Table 2. Labelled Ammonium Nitrogen
(Expressed as % of added labelled-N)

Week #	pH	mV	Mean
2	7	-100	2.63
2	5	-100	3.00
3	7	-100	2.63
3	5	-100	5.31
4	7	-100	3.13
4	7	+100	4.19
4	5	-100	6.06
4	5	+100	6.13
5	7	-100	4.94
5	7	+100	6.06
5	5	-100	10.38
5	5	+100	7.00
6	7	-100	4.38
6	7	+100	8.00
6	7	+300	9.56
6	5	-100	6.94
6	5	+100	6.50
6	5	+300	9.50

immobilized N in the decomposition of ^{15}N labelled rice straw is mineralized in the first six weeks of incubation. That suggests a rapid decline in the residual effect of rice straw application on the N availability. The labelled N unaccounted for ranged between 2 and 24 %. These results are comparable to those obtained by Reddy and Patrick (1979) who used labelled $(NH_4)_2SO_4$ instead. The N loss from the system at pH 7 is apparently due to volatilization at greater redox potential and due to both volatilization and denitrification under lower redox conditions.

5. CORRELATIONS BETWEEN NITROGEN FORMS

Table 3 shows the correlation between N_2 fixation rates and the analyzed N forms at pH 7. The degree of association decreased in the following order: organic N > labelled organic N > labelled NH_4^+-N > NH_4^+-N > NO_3^--N. The correlations between N_2 fixation and organic N, labelled organic N, NO_3^--N and labelled NH_4^+-N were highly significant. The correlation between N_2 fixation and NH_4^+-N was not significant. The correlation between N_2 fixation and NO_3^--N, although highly significant was negative (R = -0.638). Organic N and NH_4^+-N were highly correlated with their labelled fractions (R = 0.688) and R = 0.665, respectively). The correlation between total NH_4^+-N and NO_3^--N was low (R = -0.244).

The correlations between N_2 fixation and the N forms investigated at pH 5 are shown in Table 4. The degree of association decreased in the following order: organic N > labelled organic N > NH_4^+-N > labelled NH_4^+-N > NO_3^--N. At pH 5, the association between N_2 fixation and NH_4^+-N was higher than that between N_2 fixation and labelled NH_4^+-N (R = 0.306 and R = 0.253, respectively). The correlation between N_2 fixation and organic N, labelled organic N, NO_3^--N and NH_4^+-N were highly significant. The correlation between N_2 fixation and labelled NH_4^+-N was also significant. At pH 5, organic N and NH_4^+-N were highly correlated with their labelled fractions (R = 0.748 and

Table 3. Pearson Correlation Coefficients Between Nitrogen Forms at pH 7 (N = 84)

	Organic Nitrogen	Labelled Organic Nitrogen	Nitrate Nitrogen	Ammonium Nitrogen	Labelled Ammonium Nitrogen	Nitrogen Fixation
Organic Nitrogen	1.00000 0.0000[a]	0.68849 0.0001	-0.59358 0.0001	-0.05363 0.6280	-0.07335 0.5073	0.67468 0.0001
Labelled Organic Nitrogen	0.68849 0.0001	1.00000 0.0000	-0.43847 0.0001	-0.15985 0.1464	-0.01949 0.86036	0.49245 0.0001
Nitrate Nitrogen	-0.59359 0.0001	-0.43847 0.0001	1.00000 0.0000	-0.24379 0.0254	-0.29918 0.0057	-0.63792 0.0001
Ammonium Nitrogen	-0.05363 0.6820	-0.15985 0.1464	-0.24379 0.0254	1.00000 0.0000	0.66509 0.0001	0.20246 0.0647
Labelled Ammonium Nitrogen	-0.07335 0.5073	-0.01949 0.8603	-0.29918 0.0057	0.66509 0.0001	1.00000 0.0000	0.26529 0.0147
Nitrogen Fixation	0.67468 0.0001	0.49245 0.0001	-0.63792 0.0001	0.20246 0.0647	0.26529 0.0147	1.00000 0.0000

[a]Probability of >R.

Table 4.. Pearson Correlation Coefficients Between Nitrogen Forms at pH 5 (N = 84)

	Organic Nitrogen	Labelled Organic Nitrogen	Nitrate Nitrogen	Ammonium Nitrogen	Labelled Ammonium Nitrogen	Nitrogen Fixation
Organic Nitrogen	1.00000 0.0000[a]	0.74755 0.0001	-0.39756 0.0002	-0.12187 0.2694	-0.23648 0.0303	0.53073 0.0001
Labelled Organic Nitrogen	0.74755 0.0001	1.00000 0.0000	-0.36831 0.0006	-0.07308 0.5089	-0.19626 0.0736	0.48753 0.0001
Nitrate Nitrogen	-0.39756 0.0002	-0.36831 0.0006	1.00000 0.0000	-0.39281 0.0002	-0.48232 0.0001	-0.71138 0.0001
Ammonium Nitrogen	-0.12187 0.2694	-0.07308 0.5089	-0.39281 0.0002	1.00000 0.0000	0.75006 0.0001	0.30554 0.0047
Labelled Ammonium Nitrogen	-0.23648 0.0303	-0.19626 0.0736	-0.48232 0.0001	0.75006 0.0001	1.00000 0.0000	0.25296 0.0203
Nitrogen Fixation	0.53073 0.0001	0.48753 0.0001	-0.71138 0.0001	0.30554 0.0047	0.25296 0.0203	1.00000 0.0000

[a]Probability of >R.

R = 0.750, respectively). The degree of association between NH_4^+-N and NO_3^--N was low (R = 0.393).

The high correlation between N_2 fixation and organic N reflects the role of rice straw as energy source. The effect of inorganic N on N_2 fixation is well documented but remains controversial. Daeash and Mortenson (1972), Tubb and Postgate (1973), and Casselman et al. (1981) claimed that inorganic N suppresses the nitrogenase activity. Yoshida et al. (1973) reported nearly complete suppression of nitrogenase activity with an amendment of 160 ppm of inorganic N in rice soils. Teal et al. (1979) stated that great levels of NH_4^+-N in marsh soils brought about by fertilizer application inhibited bacterial N_2 fixation in the soil. Casselman et al. (1987) reported an inverse relationship between nitrogenase activity and extractable NH_4^+-N in soils, with almost complete lack of nitrogenase activity in soils containing more than 20 ppm of extractable NH_4^+-N. According to these authors other forms of inorganic N did not cause inhibition. However, Kalininskaya et al. (1973), Rao (1976), and Matsuguchi (1979) reported a stimulative effect of inorganic N on heterotrophic N_2 fixation. The negative correlation between NH_4^+-N and NO_3^- is due to the fact that these two N fractions are formed under opposite conditions.

6. PRACTICAL IMPLICATIONS OF RESEARCH TO FLOODED RICE SYSTEMS

Given the complexity of the heterotrophic N_2-fixing system in flooded systems, field researchers should investigate several parameters simultaneously. The manipulation of the soil factors appears to be the easiest. The optimum rate and timing of N fertilizer application may alleviate the inhibitive action of excess inorganic N. Additional research should be carried out on the practical use of compatible N fertilizer in heterotrophic N_2 fixation. Application of P, Ca, and Mo may stimulate the activity of heterotrophic N_2 fixers, especially in acidic soils. Breeding of high yielding rice varieties with high N_2-fixing capacity will be a great step forward. The incorporation of plant residues not only

improves the physical properties but also increases the N storage capacity of the soil thus preventing additional N loss from the system.

SUMMARY AND CONCLUSIONS

Laboratory-incubation studies were conducted to evaluate the magnitude and rate of heterotrophic N_2 fixation and N mineralization as influenced by controlled redox potential and pH. The Crowley silt loam soil was amended with 0.50 % labelled rice straw and incubated for six weeks in the dark. Four redox potential treatments (-100, +100, +300, and +500 mV) were factorially combined with two pH treatments (pH 7 and pH 5). At weekly intervals, soil suspension samples were withdrawn and analysed for N_2 fixation using the acetylene reduction assay, total organic N, extractable ammonium, and NO_3^--N, labelled organic N, and labelled extractable ammonium.

The greatest production of ethylene was recorded in the -100 mV treatment (most reduced) at all weekly samplings. The lowest was recorded at +500 mV (the most oxidized treatment).At pH 7, the rate of ethylene production decreases as the redox potential increases (-100, +100, +300, +500 mV) in the following order: 7.869, 7.140, 4.959, and 3.552 μmoles C_2H_4/kg soil·week. This corresponds to 7.505 and 4.256 μmoles C_2H_4/kg soil·week under anaerobic and aerobic conditions, respectively. At pH 5, in order of increasing redox potentials, the rates of ethylene production are 5.631, 4.486, 3.384, and 2.625 μmoles C_2H_4/kg soil·week. This corresponds to 5.059 and 3.005 μmoles C_2H_4/kg soil·week under anaerobic and aerobic conditions, respectively. The rate of production of ethylene was about twice as great under anaerobic conditions (6.282 μmoles C_2H_4/kg soil·week) as under aerobic conditions (3.630 μmoles C_2H_4/kg soil·week). At the end of the experiment, the production of ethylene at -100 mV and pH 7 (5.341 μmoles/kg soil·week) was significantly greater than that of any other treatment. The average amount of N fixed was 10 kg N_2/ha·year. From the second week of incubation the mineralization rate decreased with increase in redox potential at both pH values. The rate of extractable ammonium release decreased as the redox potential increased at both pH values. The nitrification rate increased with increase in redox

potential. It is significantly greater at pH 7 than pH 5. A large portion of the labelled organic N (77 to 96 %) remained in the organic form. In the fifth week of incubation the amount of labelled organic mineralized at pH 5 was about twice that mineralized at pH 7 (1.66 mg ^{15}N/Kg soil against 0.79 mg ^{15}N/Kg soil, respectively). The labelled ammonium released ranged from 2.63 % to 10.38 % of the total labelled N. A significant redox potential -pH interaction was found in the amounts of labelled ammonium released. The degree of correlation between N_2 fixation and other N sources is in the following order: organic N > labelled organic N > NH_4^+-N > labelled NH_4^+-N > NO_3^--N. Organic N and NH_4^+-N were highly correlated with their labelled fractions (R = 0.750 and R = 0.873, respectively.)

Given the complexity of the heterotrophic N_2-fixing system in flooded systems, field researchers should investigate several parameters simultaneously. The manipulation of the soil factors appears to be the easiest. The optimum rate and timing of N fertilizer application may alleviate the inhibitive action of excess inorganic N. Additional research should be carried out on the practical use of compatible N fertilizer in heterotrophic N_2 fixation. Application of P, Ca, and Mo may stimulate the activity of heterotrophic N_2 fixers, especially in acidic soils. Breeding of high yielding rice varieties with high N_2-fixing capacity will be a great step forward. He incorporation of plant residues not only improves the physical properties but also increases the N storage capacity of the soil thus preventing additional N loss from the system.

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APPENDIX A: SAMPLE CALCULATIONS FOR ETHYLENE PRODUCTION

$$\frac{PV}{T} = \frac{P'V'}{T'}$$

where P = actual pressure, at

T = actual temperature, °C

P' = standard pressure, 760 mm

T' = standard temperature, °C

X = volume of C_2H_4 used for the standard

X' = volume of C_2H_4 used for the standard at

standard temperature and pressure

$$\frac{763.4 \text{ mm} \times 25 \text{ µl}}{273° + 30°} = \frac{760 \times V'}{273°}$$

$$= \frac{763.4 \text{ mm} \times 25 \text{ µl}}{303°} = \frac{760 \text{ mm } V'}{273°}$$

$$V = \frac{763.4 \text{ mm} \times 25 \text{ µl} \times 273°}{303° \times 760 \text{ mm}} = \frac{5210205}{230280}$$

= 22.6329 µl of C_2H_4 (at standard temperature and pressure = STP)

1 mole of any gas at STP = 22, 4 l

Molar concentration of C_2H_4 in the bottle

$$\frac{22.6329}{22{,}400 \text{ ml}} = \frac{XM}{1M}$$

$$X = \frac{0.0226329 \text{ ml}}{22{,}400 \text{ ml}} = 0.00000101 \text{ M of } C_2H_4 \text{ in}$$

20 µl C_2H_4 injected in the bottle (1054 cc)

$$X = 1.01 \times 10^{-6} M\, C_2H_4$$

Concentration of C_2H_4 in moles/ml

$$\frac{1.01 \times 10^{-6} M}{1054 \text{ ml}} = 9.583 \times 10^{-3} \times 10^{-6} = 9.583 \times 10^{-6} \text{ moles } C_2H_4/\text{ml}$$

$$\frac{9.593 \times 10^{-10}}{1000\ \mu l} \times \frac{X}{20}$$

$$X = \frac{9.583 \times 10^{-10} \times 20}{1000} = 0.19166 \times 10^{-10} = 1.9166 \times 10^{-11}\, MC_2H_4$$

STANDARD CURVE

		Peak Height
100 µl C_2H_4 from 1054 ml bottle =	9.6×10^{-11} moles C_2H_4	15
200	19.2×10^{-11}	30
300	28.8×10^{-11}	57
400	38.4×10^{-11}	64
500	48.0×10^{-11}	79

Now we can interpret peak heights into moles of ethylene from the standard curve (Figure 29).

$$XM = \frac{X \text{ moles } C_2H_4}{\mu l \text{ injected}} \times \frac{1000\ \mu l/ml}{1} \times \frac{240 \text{ ml}}{1} \text{ (vol. of mason jar)}$$

$$= \frac{X \text{ moles } C_2H_4}{500\ \mu l} \text{ (from curve)} \times \frac{1000\ \mu l/ml}{1} \times \frac{240 \text{ ml}}{1}$$

Reference: Agronomy 4056, Soil Microbiology class notes.

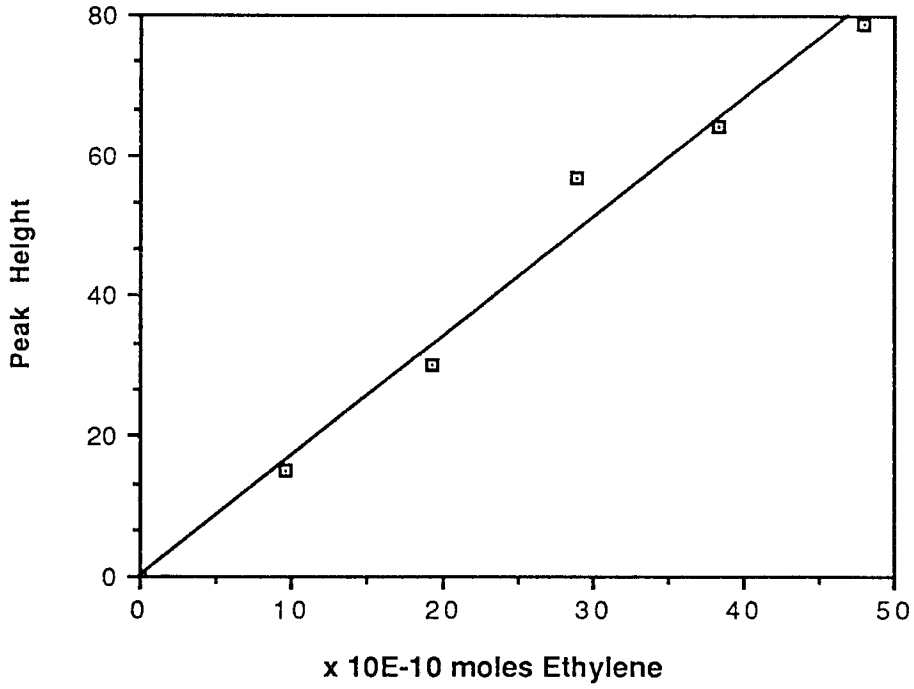

Figure 29: Acetylene reduction standard curve.

$$= \frac{3.0 \times 10^{-10} \text{X moles } C_2H_4}{500\ \mu l} \times \frac{1000\ \mu l/ml}{1} \times \frac{240\ ml}{1} = 1441.04^{-10} \text{ moles } C_2H_4$$

$$= 1.44 \times 10^{-7} \text{ moles } C_2H_4 / \text{jar}$$

ETHYLENE PRODUCTION/KG·WEEK

$$\frac{\text{Production of Ethylene/jar x 1000 g}}{\text{Weight dry soil (g)}}$$

$$= \frac{1.44 \times 10^{-7} \text{ moles } C_2H_4}{23.4 \text{ g}} \times 1000 \text{ g} = \begin{matrix} 61.54 \times 10^{-7} \text{ moles } C_2H_4/\text{kg/wk} \\ \text{or } 6.15 \text{ µmoles } C_2H_4/\text{kg/wk} \end{matrix}$$

Using the C_2H_4:N_2 theoretical ratio (3:1) the N_2 fixation would be:

$$\frac{6.15 \text{ µmoles } C_2H_4}{3} = 3.1 \text{ µmoles } N_2/\text{kg/wk}$$

Quantity of N fixed/ha/year:

If we consider the weight of a hectare furrow-slice equals 2,250,000 kg, 52 weeks/year and the average fixation rate 2.04 µmoles N_2/kg/week

$$(\frac{10.10 + 2.11}{2} \text{ µmoles } C_2H_4/\text{kg/wk}),$$

the quantity of fixed N/ha/year can be estimated using the following reasoning.

$$\frac{3.1 \text{ µmoles } N_2/\text{kg/week x } 2{,}250{,}000 \text{ kg x } 52 \text{ weeks}}{10^{-6}}$$

$$= 362.7 \text{ moles } N_2/\text{ha/year}$$

$$1 \text{ mole } N_2 = 28 \text{ g}$$

$$3.62.7 \text{ moles } N_2 = \frac{362.7 \text{ moles x } 28 \text{ g}}{10^3 \text{ g}} = 10.16 \text{ kg/ha/year}$$

APPENDIX B. SAMPLE CALCULATIONS FOR SOIL N CONTENT:

Inorganic N (NH_4^+ or NO_3^-)

Sample #

1	1.50 ml	0.0107 N HCl
2	2.50 ml	
3	2.00 ml	
	6.00 ml	

$$\frac{6 \text{ ml}}{3} = 2.00 \text{ ml of } 0.0107 \text{ N HCl}$$

Dry weight soil = 10 g

Inorganic - N in soil

$$\text{mg N/kg} = \text{ml HCl - Blk} \times \text{N HCl} \times \frac{14 \text{ mg N}}{\text{meq}} \times \frac{1000 \text{ µg N}}{\text{mg}} \times \frac{1}{10 \text{ g soil}}$$

where

ml HCl is amount of 0.0107 N HCl used for titration

Blk = amount of 0.0107 N HCl used for blank

$$= 2.00 \times 0.0107 \times \frac{14}{1} \times 1000 \times \frac{1}{2g} = 29.96 \text{ mg N/kg of soil}$$

$$\text{Total N in straw} = \text{ml HCl - Blk} \times \text{N HCl} \times \frac{14 \text{ mg N}}{\text{meq}} \times 1000 \times \frac{1}{0.1 \text{ g straw}}$$

N HCl in this case = 0.01083

$$= 5.12 \times 0.01083 \times \frac{14}{1} \times 1000 \times \frac{1}{0.1}$$

$$= 7{,}762.9 \text{ mg N/kg}$$

SAMPLE CALCULATIONS OF ATOM-PERCENT ^{15}N

$$\text{Atom-percent } ^{15}N = \frac{100}{2\,R+1}$$

Mass/charge 28 (m/e 28)

(Average peak height x multiplier for 28) - Blank for 28

Example

$$\frac{45 + 44.5}{2} = 44.75$$

$44.75 \times 300 = 13{,}425$

$13{,}425 - 9 = 13{,}416$

Mass/charge 29 (m/e 29)

$$\frac{67 + 67}{2} = 67$$

$67 \times 10 = 670$
$670 - 15 = 655$

$$R = \frac{^{14}N^{14}N}{^{15}N^{14}N} = \frac{28}{29} = \frac{13416}{655} = 20.482$$

$$\text{Atom-percent } ^{15}N = \frac{100}{[2\,(20.482) + 1]} = \frac{100}{41.9648} = 2.384$$

Atom-% ^{15}N excess = Atom % ^{15}N - Natural background ^{15}N concentration

On the mass spectrometer used the natural background concentration was 0.357. So the atom-% ^{15}N excess in this case will be 2.384 - 0.357 = 2.027.

$$N^{15} \text{ content } (\text{mg } ^{15}N/\text{kg}) = \text{mg N/kg soil} \times \frac{\text{Atom percent } ^{15}N \text{ excess}}{100}$$

$$= 703.52 \times \frac{2.027}{100} = 14.26$$

If the atom-% ^{15}N is greater than 5

$$\text{atom-percent } ^{15}N = \frac{100\,[29 + 2\,(30)]}{2\,(28 + 29 + 30)}$$

where 28, 29, and 30 are the ion currents corresponding to masses 28, 29, and 30, respectively.

Example:

for mass 28 $\quad \frac{(93.5 + 92}{2} \times 300) - 9 = 27{,}816$

for mass 29 $\quad \frac{(67 + 67}{2} \times 100) - 15 = 6{,}685$

for mass 30 $\quad \frac{(71 + 72}{2} \times 100) - 0 = 6{,}685$

$$\text{Atom-percent } ^{15}N = \frac{100\,[6{,}685 + 2\,(7{,}150)]}{2\,(27{,}816 + 6{,}685 + 7{,}150)} = 25.191$$

SAMPLE CALCULATION FOR ^{15}N MINERALIZATION

^{15}N added to each flask as rice straw:

$$\frac{2.0 \text{ g rice straw}}{1} \times \frac{7{,}762.9 \text{ µg N}}{\text{g straw}} \times \frac{41.210 \text{ atom \% } ^{15}\text{N excess}}{100} = 6{,}399.6 \text{ µg } ^{15}N$$

Amount of ^{15}N per kg of soil

$$\frac{6{,}399.6 \text{ mg } ^{15}N}{400 \text{ kg soil}} = 15.999 \text{ mg } ^{15}N/\text{kg dry soil}$$

^{15}N content of Individual Fractions
(Organic N, NH_{4+}-N, and NO_3^--N)

$$\frac{\text{mg N}}{\text{kg soil}} \times \frac{\text{atom } ^{15}\text{N excess}}{100} = \frac{\text{µg } ^{15}N}{\text{g dry soil}}$$

Percent ^{15}N in each fraction at each sampling time

$$= \frac{^{15}N \text{ in fraction}}{^{15}N \text{ added}}$$

Appendix C Table 1. Dry Weight of Soil Samples (g)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	23.40	19.56	24.54	22.50
	7	+100	22.00	20.51	23.56	22.02
		+300	21.60	20.61	21.40	21.20
		+500	24.00	21.91	22.00	22.61
0						
		-100	22.00	22.80	22.00	22.27
	5	+100	22.40	21.51	20.39	21.43
		+300	23.20	19.75	22.25	21.73
		+500	22.00	22.00	20.25	21.42
		-100	23.20	18.60	21.12	20.97
	7	+100	25.60	20.75	20.92	21.68
		+300	29.40	21.61	19.42	23.48
		+500	26.00	22.99	20.84	23.28
1						
		-100	21.40	23.83	22.04	22.42
	5	+100	25.60	21.63	22.01	22.08
		+300	22.10	20.65	20.21	20.99
		+500	25.70	22.61	21.65	23.32
		-100	22.00	19.61	21.81	21.14
	7	+100	22.40	20.70	21.64	21.58
		+300	19.60	21.17	19.87	20.21
		+500	23.00	21.51	22.40	22.30
2						
		-100	21.20	22.59	21.60	21.80
	5	+100	23.00	21.43	20.32	21.58
		+300	23.90	20.28	21.49	21.89
		+500	25.50	23.82	21.45	23.59

Appendix C Table 1. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	22.00	21.82	21.88	21.90
	7	+100	22.00	19.75	21.27	21.01
		+300	21.60	21.17	21.23	21.32
		+500	22.10	22.39	22.30	22.21
3						
		-100	20.40	22.44	22.15	21.66
	5	+100	23.20	21.88	21.34	22.14
		+300	18.30	19.58	21.28	19.72
		+500	24.70	22.58	22.13	23.14
		-100	21.70	21.72	23.39	22.27
	7	+100	22.90	19.56	22.84	21.77
		+300	21.60	21.70	21.10	21.47
		+500	23.10	21.71	23.46	22.76
4						
		-100	20.90	22.50	23.01	22.14
	5	+100	25.20	21.88	21.33	22.80
		+300	19.00	20.43	22.42	20.62
		+500	23.10	23.16	22.66	22.97
		-100	21.80	22.46	24.23	22.83
	7	+100	23.00	19.13	23.09	21.74
		+300	21.60	21.75	22.08	21.82
		+500	22.70	24.15	23.08	23.31
5						
		-100	21.80	24.91	24.18	23.26
	5	+100	23.40	22.45	20.42	22.09
		+300	18.00	19.57	22.78	20.12
		+500	22.70	22.83	22.84	22.79

Appendix C Table 1. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	20.40	22.36	21.92	21.56
	7	+100	22.40	20.50	20.31	21.07
		+300	22.40	21.50	20.35	21.42
		+500	22.90	23.10	21.33	22.44
6						
	5	-100	21.30	26.00	22.45	23.25
		+100	24.90	22.78	19.45	22.38
		+300	17.70	18.77	22.61	19.69
		+500	24.00	22.20	20.74	20.74

Appendix C Table 2. Peak Heights
(Chart Units)

Week #	pH	mV	R I	R II	RIII
		-100	48	45	42
	7	+100	41	39	38
		+300	35	34	32
		+500	21	20	20
0					
		-100	38	38	35
	5	+100	35	32	30
		+300	20	19	19
		+500	16	15	14
		-100	63	59	57
	7	+100	54	53	51
		+300	40	39	39
		+500	30	29	28
1					
		-100	46	46	43
	5	+100	38	37	37
		+300	26	25	23
		+500	22	20	19
		-100	72	69	68
	7	+100	68	65	63
		+300	44	42	39
		+500	35	34	31
2					
		-100	54	53	49
	5	+100	46	45	45
		+300	30	26	25
		+500	25	25	23

Appendix C Table 2. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	72	68	67
	7	+100	66	65	62
		+300	40	38	38
		+500	30	28	27
3					
		-100	48	46	46
	5	+100	32	30	29
		+300	25	25	25
		+500	22	19	19
		-100	60	59	56
	7	+100	56	54	50
		+300	37	35	32
		+500	28	28	26
4					
		-100	40	39	37
	5	+100	32	30	30
		+300	22	22	19
		+500	18	18	17
		-100	55	52	48
	7	+100	50	49	46
		+300	30	29	26
		+500	26	26	22
5					
		-100	38	38	36
	5	+100	28	27	27
		+300	24	22	22
		+500	24	20	20

Appendix C Table 2. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	42	39	34
	7	+100	30	28	28
		+300	28	26	26
		+500	21	19	19
6					
	5	-100	30	28	27
		+100	24	24	22
		+300	20	18	18
		+500	17	17	17

Appendix C Table 3. Production of C_2H_4
x10-7 moles C_2H_4/jar

Week #	pH	mV	R I	R II	RIII
		-100	1.44	1.38	1.28
	7	+100	1.25	1.20	1.16
		+300	1.07	1.04	0.98
		+500	0.63	0.60	0.60
0					
		-100	1.16	1.16	1.07
	5	+100	1.07	0.97	0.912
		+300	0.61	0.58	0.58
		+500	0.48	0.47	0.41
		-100	1.94	1.94	1.74
	7	+100	1.65	1.62	1.56
		+300	1.22	1.19	1.19
		+500	0.91	0.89	0.85
1					
		-100	1.41	1.41	1.31
	5	+100	1.17	1.13	1.13
		+300	0.80	0.77	0.70
		+500	0.67	0.61	0.58
		-100	2.22	2.12	2.07
	7	+100	2.07	2.00	1.93
		+300	1.34	1.29	1.19
		+500	1.07	1.04	0.94
2					
		-100	1.65	1.62	1.52
	5	+100	1.41	1.38	1.38
		+300	0.912	0.80	0.77
		+500	0.77	0.77	0.70

Appendix C Table 3. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	2.22	2.07	2.06
	7	+100	2.03	1.98	1.90
		+300	1.22	1.16	1.16
		+500	0.92	0.85	0.81
3					
		-100	1.44	1.42	1.42
	5	+100	0.98	0.92	0.88
		+300	0.77	0.77	0.77
		+500	0.69	0.58	0.58
		-100	1.74	1.81	1.71
	7	+100	1.71	1.65	1.54
		+300	1.13	1.07	0.98
		+500	0.85	0.85	0.80
4					
		-100	1.22	1.20	1.13
	5	+100	0.98	0.92	0.92
		+300	0.68	0.68	0.58
		+500	0.55	0.55	0.52
		-100	1.68	1.59	1.44
	7	+100	1.54	1.51	1.41
		+300	0.92	0.89	0.80
		+500	0.80	0.80	0.67
5					
		-100	1.16	1.16	1.10
	5	+100	0.85	0.82	0.82
		+300	0.73	0.68	0.68
		+500	0.73	0.61	0.61

Appendix C Table 3. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	1.28	1.19	1.04
	7	+100	0.91	0.85	0.85
		+300	0.85	0.80	0.80
		+500	0.63	0.58	0.58
6					
	5	-100	0.91	0.85	0.82
		+100	0.75	0.75	0.68
		+300	0.61	0.55	0.55
		+500	0.52	0.52	0.52

Appendix C Table 4. Production of C_2H_4
(10^{-10} moles C_2H_4)

Week #	pH	mV	R I	R II	RIII
		-100	3.00	2.88	2.66
	7	+100	2.60	2.50	2.42
		+300	2.22	2.16	2.04
		+500	1.32	1.26	1.26
0					
		-100	2.42	2.42	2.22
	5	+100	2.22	2.02	1.90
		+300	1.28	1.20	1.20
		+500	1.0	0.98	0.86
		-100	4.04	3.78	3.62
	7	+100	3.44	3.38	3.26
		+300	2.54	2.48	2.48
		+500	1.90	1.86	1.78
1					
		-100	2.94	2.94	2.72
	5	+100	2.44	2.36	2.36
		+300	1.66	1.60	1.46
		+500	1.40	1.28	1.20
		-100	4.62	4.42	4.32
	7	+100	4.32	4.16	4.02
		+300	2.80	2.68	2.48
		+500	2.22	2.16	1.96
2					
		-100	3.44	3.38	3.16
	5	+100	2.94	2.88	2.88
		+300	1.90	1.66	1.60
		+500	1.60	1.60	1.46

Appendix C Table 4. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	4.62	4.32	4.30
	7	+100	4.22	4.12	3.96
		+300	2.54	2.42	2.42
		+500	1.92	1.78	1.68
3					
		-100	3.00	2.96	2.96
	5	+100	2.04	1.92	1.84
		+300	1.60	1.60	1.60
		+500	1.40	1.20	1.20
		-100	3.84	3.78	3.56
	7	+100	3.56	3.44	3.20
		+300	2.36	2.22	2.04
		+500	1.78	1.78	1.66
4					
		-100	2.54	2.50	2.36
	5	+100	2.04	1.92	1.92
		+300	1.42	1.42	1.20
		+500	1.14	1.14	1.08
		-100	3.50	3.32	3.00
	7	+100	3.20	3.14	2.94
		+300	1.92	1.86	1.66
		+500	1.66	1.66	1.40
5					
		-100	2.42	2.42	2.30
	5	+100	1.78	1.70	1.70
		+300	1.52	1.42	1.42
		+500	1.52	1.28	1.28

Appendix C Table 4. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	2.66	2.48	2.16
	7	+100	1.90	1.78	1.78
		+300	1.78	1.66	1.66
		+500	1.32	1.20	1.20
6					
	5	-100	1.90	1.78	1.70
		+100	1.57	1.57	1.42
		+300	1.28	1.14	1.14
		+500	1.08	1.08	1.08

Appendix C Table 5. Nitrogen Fixation
(μmoles C_2H_4/kg/week)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	6.154	7.055	5.216	6.142
	7	+100	5.682	5.851	4.924	5.486
		+300	4.954	5.046	4.579	4.860
		+500	2.625	2.738	2.727	2.697
0						
		-100	5.273	5.088	4.864	5.075
	5	+100	4.777	4.510	4.473	4.587
		+300	2.629	2.937	2.607	2.724
		+500	2.182	2.136	2.025	2.114
		-100	8.362	10.430	8.239	9.010
	7	+100	6.445	7.807	7.457	7.236
		+300	4.150	5.507	6.128	5.261
		+500	3.500	3.871	4.079	3.817
1						
		-100	6.589	5.917	5.944	6.150
	5	+100	4.570	5.224	5.134	4.976
		+300	3.620	3.729	3.810	3.720
		+500	2.607	2.689	2.679	2.661
		-100	10.091	10.811	9.491	10.131
	7	+100	9.241	9.662	8.919	9.274
		+300	6.837	6.094	5.989	6.307
		+500	4.652	4.835	4.196	4.561
2						
		-100	7.174	7.559	7.480	7.404
	5	+100	6.130	6.440	6.791	6.454
		+300	3.816	3.945	3.583	3.781
		+500	3.020	3.219	3.263	3.167

Appendix C Table 5. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	10.091	9.487	9.415	9.664
	7	+100	9.227	10.025	8.933	9.395
		+300	5.648	5.479	5.464	5.530
		+500	4.163	3.796	3.632	3.864
3						
		-100	7.059	6.328	6.411	6.599
	5	+100	4.224	4.205	4.124	4.184
		+300	4.208	3.933	3.618	3.920
		+500	2.794	2.569	2.621	2.661
		-100	8.018	8.333	7.311	7.887
	7	+100	7.467	8.436	6.743	7.549
		+300	5.231	4.931	4.645	4.936
		+500	3.680	3.915	3.410	3.668
4						
		-100	5.837	5.333	4.911	5.360
	5	+100	3.889	4.205	4.313	4.136
		+300	3.579	3.328	2.587	3.165
		+500	2.895	2.692	2.319	2.635
		-100	7.706	7.079	5.943	6.909
	7	+100	6.696	7.893	6.107	6.899
		+300	4.259	4.092	3.623	3.991
		+500	3.524	3.313	2.903	3.247
5						
		-100	5.321	4.657	4.549	4.842
	5	+100	3.632	3.653	4.016	3.767
		+300	4.056	3.475	2.985	3.505
		+500	3.216	2.672	2.671	2.853

Appendix C Table 5. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	5.818	5.454	4.753	5.341
	7	+100	4.136	4.304	3.978	4.139
		+300	3.935	3.779	3.768	3.827
		+500	3.846	2.590	2.601	3.012
6						
	5	-100	4.461	3.788	3.702	3.984
		+100	3.232	3.341	3.330	3.301
		+300	3.389	2.810	2.414	2.871
		+500	2.291	2.278	2.277	2.282

Appendix C Table 6. Organic Nitrogen
(ml 0.01083 NH_2SO_4 required for titration)

Week #	pH	mV	R I	R II	RIII
0	7	-100	8.30	8.42	8.68
		+100	8.34	8.38	8.46
		+300	8.16	8.20	8.20
		+500	8.12	8.20	8.10
	5	-100	8.48	8.34	8.54
		+100	8.30	8.30	8.42
		+300	8.24	8.30	8.20
		+500	8.16	8.10	8.28
1	7	-100	9.42	9.50	9.34
		+100	9.30	9.42	0.42
		+300	9.50	9.30	9.52
		+500	9.00	8.98	9.36
	5	-100	9.00	9.20	9.46
		+100	8.90	9.00	9.46
		+300	8.82	8.92	9.30
		+500	8.67	8.70	9.38
2	7	-100	9.56	9.62	9.62
		+100	9.46	9.50	9.54
		+300	9.50	9.50	9.42
		+500	9.20	9.10	9.34
	5	-100	9.48	9.50	9.46
		+100	9.32	9.34	9.52
		+300	9.36	9.28	9.46
		+500	9.22	9.10	9.38

Appendix C Table 6. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	9.42	9.30	9.56
	7	+100	9.32	9.22	9.32
		+300	9.18	9.18	9.20
		+500	8.78	9.10	8.98
3					
		-100	8.64	8.60	8.76
	5	+100	8.70	8.60	8.56
		+300	8.64	8.52	8.46
		+500	8.30	8.28	8.44
		-100	9.22	9.08	9.30
	7	+100	8.94	8.76	8.84
		+300	8.48	8.36	8.60
		+500	8.24	8.16	8.40
4					
		-100	8.64	8.70	8.72
	5	+100	8.42	8.32	8.44
		+300	8.10	8.02	8.14
		+500	8.24	7.90	7.94
		-100	8.64	8.52	8.84
	7	+100	8.24	8.08	8.42
		+300	8.00	7.92	8.10
		+500	8.10	8.12	7.84
5					
		-100	7.78	7.56	8.18
	5	+100	7.36	7.18	7.76
		+300	7.40	7.18	7.54
		+500	7.22	7.00	7.10

Appendix C Table 6. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	8.92	8.52	8.64
	7	+100	8.64	8.24	8.18
		+300	8.12	8.02	8.00
		+500	7.98	7.88	7.76
6					
		-100	7.68	8.20	8.14
	5	+100	7.81	7.96	8.00
		+300	7.42	7.78	7.80
		+500	7.42	7.48	7.52

Appendix C Table 7. Organic Nitrogen
(mg N/kg soil)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	629.22	638.32	658.03	641.86
	7	+100	632.15	635.29	641.35	636.26
		+300	618.61	621.64	621.64	620.63
		+500	615.58	621.64	614.06	617.09
0						
		-100	642.87	632.26	647.59	640.41
	5	+100	629.22	629.22	638.32	632.25
		+300	624.67	629.22	621.64	625.18
		+500	618.58	614.06	627.71	620.12
		-100	714.13	720.20	708.07	714.13
	7	+100	707.03	714.13	714.13	711.76
		+300	720.25	705.03	721.71	715.66
		+500	682.29	680.77	709.58	690.88
1						
		-100	682.29	697.36	717.16	698.94
	5	+100	674.81	682.29	717.16	691.42
		+300	668.72	676.23	705.03	683.33
		+500	657.29	659.55	711.10	675.98
		-100	717.92	729.29	729.29	725.50
	7	+100	717.16	720.20	723,23	720.20
		+300	720.56	720.20	714.13	718.30
		+500	697.45	689.87	708.07	698.46
2						
		-100	718.68	720.20	717.16	718.68
	5	+100	706.55	708.07	721.71	712.11
		+300	709.58	703.52	717.16	710.09
		+500	698.97	691.00	711.10	700.36

Appendix C Table 7. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	714.13	705.03	724.74	714.63
	7	+100	706.55	698.97	706.55	704.02
		+300	695.94	695.94	697.45	696.44
		+500	665.61	689.87	680.77	678.75
3						
		-100	655.00	651.97	664.10	657.02
	5	+100	659.55	651.97	664.10	653.48
		+300	655.00	645.90	641.35	647.41
		+500	629.67	627.71	639.84	632.41
		-100	698.97	688.35	705.03	697.45
	7	+100	677.74	664.10	670.16	670.67
		+300	642.87	633.94	651.97	642.93
		+500	624.67	618.60	636.80	626.69
4						
		-100	655.00	659.55	661.06	658.54
	5	+100	638.32	630.74	639.84	636.30
		+300	614.06	608.00	617.09	613.05
		+500	624.67	598.90	601.93	608.50
		-100	655.00	645.90	670.16	657.02
	7	+100	624.67	612.54	638.32	625.18
		+300	606.48	600.42	614.06	606.99
		+500	614.06	615.58	594.35	607.99
5						
		-100	589.80	573.12	620.13	594.35
	5	+100	557.96	544.32	588.28	563.52
		+300	560.99	544.32	571.61	559.00
		+500	547.35	530.67	538.25	538.76

Appendix C Table 7. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	676.23	645.90	655.00	659.04
	7	+100	655.00	624.67	620.13	633.27
		+300	615.58	608.00	606.48	610.02
		+500	604.96	597.38	588.29	596.88
6						
	5	-100	582.22	560.99	583.74	575.65
		+100	530.67	541.28	606.48	559.48
		+300	562.51	530.67	538.25	543.81
		+500	538.25	539.77	545.83	541.28

Appendix C Table 8. Ammonium Nitrogen
(ml 0.01083 NH_2SO_4 required for titration)

Week #	pH	mV	R I	R II	RIII
		-100	0.14	0.12	0.16
	7	+100	0.12	0.10	0.14
		+300	0.10	0.10	0.10
		+500	0.10	0.10	0.10
0					
		-100	0.24	0.10	0.18
	5	+100	0.12	0.10	0.12
		+300	0.12	0.08	0.14
		+500	0.10	0.10	0.12
		-100	0.12	0.10	0.12
	7	+100	0.12	0.10	0.10
		+300	0.12	0.10	0.08
		+500	0.10	0.06	0.08
1					
		-100	0.46	0.50	0.50
	5	+100	0.14	0.10	0.14
		+300	0.10	0.10	0.12
		+500	0.10	0.10	0.10
		-100	1.18	1.20	1.20
	7	+100	0.25	0.30	0.28
		+300	0.15	0.16	0.14
		+500	0.17	0.16	0.16
2					
		-100	3.00	2.68	3.20
	5	+100	0.40	0.30	0.36
		+300	0.40	0.28	0.38
		+500	0.10	0.16	0.30

Appendix C Table 8. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	3.00	2.20	3.40
	7	+100	0.52	0.45	0.60
		+300	0.25	0.28	0.34
		+500	0.20	0.24	0.26
3					
		-100	4.58	3.50	5.00
	5	+100	0.62	0.72	0.70
		+300	0.26	0.28	0.54
		+500	0.30	0.26	0.30
		-100	5.68	5.26	5.20
	7	+100	0.74	0.80	0.82
		+300	0.56	0.30	0.40
		+500	0.30	0.30	0.40
4					
		-100	6.30	6.50	6.58
	5	+100	0.76	0.96	0.80
		+300	0.58	0.58	0.60
		+500	0.36	0.40	0.40
		-100	6.40	4.48	6.20
	7	+100	0.82	0.70	0.90
		+300	0.64	0.60	0.72
		+500	0.50	0.52	0.66
5					
		-100	8.50	8.32	8.70
	5	+100	1.20	1.32	1.10
		+300	0.60	0.76	0.62
		+500	0.30	0.48	0.48

Appendix C Table 8. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	8.00	7.20	8.22
	7	+100	1.02	1.20	1.22
		+300	0.70	0.80	0.74
		+500	0.56	0.80	0.60
6					
	5	-100	11.58	12.42	11.22
		+100	2.60	2.00	2.20
		+300	0.80	0.82	1.00
		+500	0.48	0.56	0.52

Appendix C Table 9. Ammonium Nitrogen
(mg N/kg of soil)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	0.907	0.930	0.989	0.942
	7	+100	0.827	0.739	0.900	0.822
		+300	0.702	0.736	0.708	0.715
		+500	0.632	0.692	0.689	0.671
0						
		-100	1.654	0.665	1.240	1.186
	5	+100	0.812	0.705	0.892	0.803
		+300	0.784	0.614	0.954	0.784
		+500	0.689	0.689	0.898	0.754
		-100	0.784	0.815	0.861	0.820
	7	+100	0.712	0.731	0.725	0.723
		+300	0.627	0.702	0.622	0.650
		+500	0.583	0.396	0.582	0.520
1						
		-100	3.259	3.181	3.440	2.132
	5	+100	0.829	0.698	0.964	0.830
		+300	0.686	0.734	0.900	0.773
		+500	0.600	0.671	0.700	0.657
		-100	12.405	9.280	8.340	10.008
	7	+100	1.192	2.197	1.962	1.784
		+300	1.160	1.146	1.068	1.135
		+500	1.121	1.128	1.083	1.111
2						
		-100	21.456	17.988	22.462	20.635
	5	+100	2.637	2.123	2.686	2.482
		+300	2.538	2.093	2.681	2.437
		+500	0.595	1.018	2.121	1.245

Appendix C Table 9. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	20.790	15.287	23.561	19.879
	7	+100	3.584	3.455	4.277	3.772
		+300	1.755	2.005	2.428	2.062
		+500	1.372	1.625	1.768	1.588
3						
		-100	34.040	23.648	34.226	30.638
	5	+100	4.052	6.256	4.973	5.094
		+300	2.154	2.168	3.848	2.723
		+500	1.842	1.746	2.055	1.881
		-100	39.687	36.718	33.708	36.704
	7	+100	4.000	6.200	5.443	5.214
		+300	3.931	2.096	2.874	2.967
		+500	1.970	2.095	2.585	2.217
4						
		-100	45.704	43.800	43.358	44.287
	5	+100	4.573	6.652	5.687	5.637
		+300	4.628	4.304	4.058	4.330
		+500	2.360	2.615	2.676	2.550
		-100	44.512	30.240	38.797	37.850
	7	+100	5.401	5.548	5.910	5.620
		+300	4.492	4.183	4.944	4.540
		+500	3.340	3.265	4.336	3.647
5						
		-100	59.118	52.235	54.553	55.302
	5	+100	7.775	8.915	8.168	8.286
		+300	5.054	5.888	4.127	5.023
		+500	2.004	3.188	3.186	2.793

Appendix C Table 9. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	59.459	48.822	56.858	55.046
	7	+100	6.904	8.875	9.108	8.296
		+300	4.738	5.642	5.513	5.298
		+500	3.708	5.250	4.265	4.408
6						
	5	-100	82.973	72.428	75.776	70.059
		+100	15.832	13.312	17.150	15.431
		+300	6.853	6.624	6.706	6.728
		+500	3.032	3.825	3.801	3.553

Appendix C Table 10. Nitrate-Nitrogen
(ml 0.01083 NH_2SO_4 required for titration)

Week #	pH	mV	R I	R II	RIII
0	7	+300	0.10	0.12	0.10
		+500	0.18	0.16	0.12
	5	+300	0.10	0.10	0.10
		+500	0.12	0.14	0.10
1	7	+300	0.20	0.10	0.14
		+500	0.20	0.14	0.16
	5	+300	0.12	0.10	0.10
		+500	0.18	0.12	0.12
2	7	+300	0.14	0.10	0.14
		+500	0.18	0.14	0.16
	5	+300	0.14	0.10	0.12
		+500	0.16	0.12	0.12
3	7	+300	0.12	0.10	0.12
		+500	0.14	0.14	0.14
	5	+300	0.10	0.10	0.12
		+500	0.16	0.12	0.14
		+300	0.24	0.22	0.24
4	7	+500	0.30	0.28	0.34
		+300	0.12	0.12	0.14
	5	+500	0.16	0.12	0.18
5	7	+300	0.40	0.44	0.46
		+500	0.46	0.50	0.60
	5	+300	0.14	0.14	0.16
		+500	0.18	0.18	0.22
6	7	+300	0.60	0.54	0.60
		+500	0.70	0.64	0.70
	5	+300	0.20	0.20	0.20
		+500	0.30	0.32	0.26

Appendix C Table 11. Nitrate-Nitrogen
(mg N/kg soil)

Week #	pH	mV	R I	R II	RIII	Mean
	7	+300	0.76	0.88	0.71	0.78
0		+500	1.14	1.11	0.83	1.03
	5	+300	0.65	0.77	0.68	0.70
		+500	0.83	0.97	0.74	0.85
	7	+300	1.09	0.70	1.09	0.96
1		+500	1.17	0.92	0.16	0.75
	5	+300	0.82	0.73	0.75	0.77
		+500	1.06	0.81	0.84	0.90
	7	+300	1.08	0.71	1.07	0.95
2		+500	1.19	0.94	1.08	1.39
	5	+300	0.89	0.75	0.85	0.83
		+500	0.95	0.76	0.85	1.13
	7	+300	0.84	0.72	0.86	0.81
3		+500	1.10	1.00	0.95	1.02
	5	+300	0.83	0.77	0.86	0.82
		+500	0.98	0.96	0.96	0.97
	7	+300	1.68	1.54	1.73	1.65
		+500	1.97	1.96	2.20	2.04
4	5	+300	0.95	0.89	0.95	0.93
		+500	1.05	1.05	1.20	1.10
	7	+300	2.81	2.76	3.16	2.91
5		+500	3.07	3.34	3.94	3.45
	5	+300	1.18	1.09	1.07	1.11
		+500	1.26	1.20	1.46	1.31
	7	+300	4.06	3.81	4.47	4.11
6		+500	4.64	4.20	4.98	4.61
	5	+300	1.71	1.61	1.34	1.55
		+500	1.90	2.19	1.90	2.00

Appendix C Table 12. Labelled Organic Nitrogen
(Atom % ^{15}N)

Week #	pH	mV	R I	R II	RIII
0	7	-100	2.690	2.369	2.629
		+100	2.719	2.346	2.607
		+300	2.709	2.365	2.646
		+500	2.663	2.368	2.657
	5	-100	2.623	2.357	2.627
		+100	2.712	2.349	2.647
		+300	2.670	2.389	2.671
		+500	2.639	2.357	2.619
1	7	-100	2.542	2.378	2.417
		+100	2.554	2.365	2.407
		+300	2.523	2.365	2.397
		+500	2.567	2.369	2.369
	5	-100	2.557	2.389	2.377
		+100	2.548	2.376	2.387
		+300	2.557	2.371	2.385
		+500	2.543	2.378	2.365
2	7	-100	2.545	2.400	2.416
		+100	2.547	2.385	2.402
		+300	2.539	2.371	2.405
		+500	2.567	2.369	2.388
	5	-100	2.545	2.411	2.411
		+100	2.548	2.393	2.395
		+300	2.544	2.384	2.381
		+500	2.539	2.381	2.375

Appendix C Table 12. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	2.542	2.435	2.517
	7	+100	2.549	2.408	2.544
		+300	2.547	2.399	2.562
		+500	2.579	2.384	2.597
3					
		-100	2.574	2.443	2.550
	5	+100	2.574	2.424	2.557
		+300	2.572	2.392	2.659
		+500	2.690	2.389	2.630
		-100	2.545	2.471	2.536
	7	+100	2.578	2.474	2.579
		+300	2.652	2.458	2.566
		+500	2.690	2.455	2.586
4					
		-100	2.545	2.478	2.570
	5	+100	2.588	2.490	2.591
		+300	2.583	2.468	2.595
		+500	2.581	2.452	2.624
		-100	2.588	2.530	2.547
	7	+100	2.698	2.508	2.590
		+300	2.695	2.499	2.580
		+500	2.691	2.486	2.582
5					
		-100	2.698	2.519	2.584
	5	+100	2.696	2.506	2.689
		+300	2.692	2.486	2.702
		+500	2.694	2.478	2.707

Appendix C Table 12. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	2.528	2.516	2.569
	7	+100	2.583	2.516	2.585
		+300	2.690	2.494	2.583
		+500	2.698	2.482	2.687
6					
		-100	2.687	2.521	2.585
	5	+100	2.812	2.508	2.575
		+300	2.815	2.500	2.582
		+500	2.803	2.467	2.587

Appendix C Table 13. Labelled Organic Nitrogen
(Atom % ^{15}N Excess)

Week #	pH	mV	R I	R II	RIII
0	7	-100	2.333	2.012	2.272
		+100	2.362	1.989	2.250
		+300	2.352	2.008	2.289
		+500	2.306	2.021	2.300
	5	-100	2.266	2.000	2.270
		+100	2.355	1.992	2.290
		+300	2.313	2.032	2.314
		+500	2.282	2.000	2.262
1	7	-100	2.185	2.021	2.060
		+100	2.197	2.008	2.052
		+300	2.166	2.008	2.040
		+500	2.210	2.012	2.012
	5	-100	2.200	2.032	2.020
		+100	2.191	2.019	2.030
		+300	2.200	2.014	2.028
		+500	2.186	2.021	2.008
2	7	-100	2.188	2.043	2.059
		+100	2.190	2.028	2.045
		+300	2.182	2.014	2.048
		+500	2.210	2.012	2.031
	5	-100	2.188	2.054	2.054
		+100	2.191	2.036	2.038
		+300	2.187	2.027	2.024
		+500	2.182	2.024	2.018

Appendix C Table 13. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	2.185	2.078	2.160
	7	+100	2.192	2.051	2.187
		+300	2.190	2.042	2.205
		+500	2.222	2.027	2.240
3					
		-100	2.217	2.086	2.193
	5	+100	2.217	2.067	2.200
		+300	2.215	2.035	2.302
		+500	2.333	2.032	2.273
		-100	2.188	2.114	2.179
	7	+100	2.221	2.117	2.222
		+300	2.295	2.101	2.209
		+500	2.333	2.098	2.229
4					
		-100	2.188	2.121	2.213
	5	+100	2.231	2.133	2.234
		+300	2.226	2.111	2.238
		+500	2.224	2.095	2.267
		-100	2.231	2.173	2.190
	7	+100	2.341	2.151	2.233
		+300	2.338	2.142	2.223
		+500	2.334	2.129	2.225
5					
		-100	2.341	2.162	2.227
	5	+100	2.339	2.149	2.332
		+300	2.335	2.129	2.345
		+500	2.337	2.121	2.350

Appendix C Table 13. (continued)

Week #	pH	mV	R I	R II	RIII
		-100	2.231	2.159	2.212
	7	+100	2.226	2.159	2.228
		+300	2.333	2.131	2.236
		+500	2.341	2.125	2.330
6					
		-100	2.330	2.164	2.228
	5	+100	2.455	2.151	2.218
		+300	2.458	2.143	2.225
		+500	2.446	2.110	2.230

Appendix C Table 14. Labelled Organic Nitrogen
(mg ^{15}N/kg soil)

Week #	pH	mV	R I	R II	RIII	Mean
0	7	-100	14.68	12.84	14.95	14.16
		+100	14.93	12.64	14.43	14.00
		+300	14.55	12.48	14.23	13.75
		+500	14.19	12.56	14.22	13.66
	5	-100	14.56	12.65	14.70	13.97
		+100	14.25	12.53	14.63	13.80
		+300	14.44	12.79	13.38	13.54
		+500	14.11	12.28	14.20	13.53
1	7	-100	15.60	14.58	14.59	14.92
		+100	15.53	14.34	14.65	14.84
		+300	15.60	14.16	14.72	14.83
		+500	15.08	13.70	14.28	14.35
	5	-100	15.10	14.17	14.49	14.59
		+100	14.79	13.89	14.56	14.41
		+300	14.71	13.62	14.30	14.21
		+500	14.37	13.33	14.28	13.99
2	7	-100	15.71	14.90	15.02	15.23
		+100	15.70	14.61	14.79	15.03
		+300	15.72	14.50	14.63	14.95
		+500	15.41	13.88	14.38	14.56
	5	-100	15.72	14.79	14.73	15.08
		+100	15.48	14.42	14.71	14.87
		+300	15.52	14.26	14.52	14.77
		+500	15.25	13.99	14.35	14.53

Appendix C Table 14. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	15.60	14.65	15.65	15.30
	7	+100	15.49	14.34	15.45	15.09
		+300	15.24	14.21	15.38	14.94
		+500	14.34	13.98	15.25	14.52
3						
		-100	14.79	13.60	14.56	14.32
	5	+100	14.52	13.48	14.28	14.09
		+300	14.51	13.14	14.76	14.14
		+500	14.69	12.76	14.54	14.00
		-100	15.29	14.55	15.38	15.07
	7	+100	15.05	14.06	14.89	14.67
		+300	14.75	13.32	14.40	14.16
		+500	14.57	12.98	14.19	13.91
4						
		-100	14.33	13.99	14.63	14.32
	5	+100	14.24	13.45	14.29	13.99
		+300	13.67	12.83	13.81	13.44
		+500	13.89	12.54	13.65	13.36
		-100	14.61	14.04	14.68	14.44
	7	+100	14.62	13.18	14.25	14.02
		+300	14.17	12.86	13.65	13.56
		+500	14.33	13.11	13.22	13.55
5						
		-100	13.81	12.39	13.81	13.34
	5	+100	13.81	12.39	13.81	12.82
		+300	13.10	11.69	13.72	12.70
		+500	12.79	11.26	12.65	12.23

Appendix C Table 14. (continued)

Week #	pH	mV	R I	R II	RIII	Mean
		-100	15.09	13.94	14.49	14.51
	7	+100	14.58	13.49	13.82	13.96
		+300	14.11	12.99	13.56	13.55
		+500	14.16	12.69	13.71	13.52
6						
		-100	13.57	13.45	13.75	12.90
	5	+100	14.54	12.98	13.45	12.70
		+300	13.63	12.64	13.16	12.34
		+500	13.76	12.12	12.71	12.24

Appendix C Table 15. Labelled Ammonium Nitrogen
(Atom % ^{15}N)

Week #	pH	mV	R I	R II	RIII
2	7	-100	1.969	5.529	7.311
	5	-100	1.196	3.693	3.251
3	7	-100	1.560	5.290	1.206
	5	-100	2.120	5.431	2.548
	7	-100	1.239	2.400	1.544
		+100	7.857	11.970	7.706
4	5	-100	2.326	3.325	2.433
		+100	19.600	17.645	33.471
	7	-100	1.630	3.730	2.058
		+100	12.392	16.579	9.157
5	5	-100	2.810	3.899	3.437
		+100	13.862	14.939	12.600
		-100	1.198	2.282	1.518
	7	+100	6.875	10.160	6.066
		+300	6.280	8.856	5.984
6					
		-100	1.683	2.014	1.729
	5	+100	4.463	9.897	7.354
		+300	4.925	6.699	6.212

Appendix C Table 16. Labelled Ammonium Nitrogen
(Atom % ^{15}N excess)

Week #	pH	mV	R I	R II	RIII
2	7	-100	1.612	5.172	6.954
	5	-100	0.839	3.336	2.894
3	7	-100	1.203	5.233	0.849
	5	-100	1.763	5.074	2.191
	7	-100	0.882	2.043	1.187
		+100	7.500	11.613	7.349
4	5	-100	1.969	2.968	2.076
		+100	19.243	17.288	33.114
	7	-100	1.573	3.373	1.701
		+100	12.035	16.222	8.800
5	5	-100	2.453	3.542	3.080
		+100	13.505	14.582	12.243
		-100	0.841	1.925	1.161
	7	+100	6.518	9.803	5.709
		+300	5.923	8.499	5.627
6					
		-100	1.326	1.657	1.327
	5	+100	4.106	9.540	6.997
		+300	4.568	6.342	5.855

Appendix C Table 17. Labelled Ammonium Nitrogen ($mg^{15}N/kg$ soil)

Week #	pH	mV	R I	R II	RIII	Mean
2	7	-100	0.20	0.48	0.58	0.42
	5	-100	0.18	0.60	0.65	0.48
3	7	-100	0.25	0.80	0.20	0.42
	5	-100	0.60	1.20	0.75	0.85
	7	-100	0.35	0.75	0.40	0.50
		+100	0.30	0.72	0.40	0.47
4	5	-100	0.90	1.30	0.70	0.97
		+100	0.88	1.15	0.90	0.98
	7	-100	0.70	1.02	0.66	0.79
		+100	0.65	0.90	0.52	0.69
5	5	-100	1.45	1.85	1.68	1.66
		+100	1.05	1.30	1.00	1.12
		-100	0.50	0.94	0.66	0.70
	7	+100	0.45	0.87	0.52	0.61
		+300	0.28	0.48	0.31	0.36
6						
	5	-100	1.10	1.20	1.04	1.11
		+100	0.65	1.27	1.20	1.04
		+300	0.31	0.42	0.39	0.37

VITA

The author was born on August 17, 1947, in Timbuctoo (Republic of Mali). After completion of his baccalaureat in Biological Sciences, he went to Russia, where he obtained his Masters Degree in Agronomy in 1974. In his home country he worked as a training officer at the Segou Rice Project. He underwent several specialized rice production and research training programs. In July 1976 he joined the West Africa Rice Development Association as an agronomist at the Special Research Project in Mopti, Mali. He served also as Consultant to the USAID and the World Bank in the Rice Development Projects funded by these organizations. He implemented three rice research stations in Republic of Guinea. In January 1984 he enrolled at Louisiana State University to pursue a Ph.D. in Agronomy.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Almoubarakou I. Toure

Major Field: Agronomy

Title of Dissertation: Effect of Controlled Redox Potential and pH on Heterotrophic Nitrogen Fixation and Mineralization in Crowley Silt-Loam Soil

Approved:

Clark W. Lindau
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Date of Examination:

November 21, 1988