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Nitrification - Denitrification Reactions in Flooded Soil.

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NITRIFICATION - DENITRIFICATION REACTIONS IN FLOODED SOIL

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Losses of applied N in flooded soils are greatly influenced by nitrification-denitrification reactions. Tracer studies using ^{15}N labelled ammonium sulfate were conducted to demonstrate the pathways of N loss in flooded soil.

The roles of NH_4^+-N diffusion, nitrification, NO_3^--N diffusion and denitrification in controlling N loss from continuously flooded soil were evaluated in independent experiments. The slow rate of NH_4^+-N diffusion from the anaerobic layer to the overlying aerobic layer, and the slow rate of nitrification in the aerobic layer indicate that these two processes are limiting steps in controlling N loss. Nitrate diffusion from the aerobic layer to the underlying anaerobic layer and denitrification in the anaerobic layer were found to proceed at faster rates and are not likely to limit N loss in a flooded soil. Increasing the concentration of O_2 in the atmosphere above the flood-water increased NH_4^+-N loss. Oxygen aided in development of a thick aerobic layer, thus increasing the zone of nitrification. The final gaseous end products of nitrification-denitrification reaction were N_2 and N_2O.

The total loss of NH_4^+-N from a soil column (15-cm depth) by nitrification-denitrification was equivalent to 12.43 g N/m^2 (124.3 kg/ha) for a 120-day incubation period when the initial concentration of NH_4^+-N was 44.84 g N/m^2 (448.4 kg/ha). Diffusion of NH_4^+-N from the

xi

anaerobic layer to the aerobic layer accounted for more than 50% of the total NH_4^+-N loss with the remainder being lost from NH_4^+-N originally present in the aerobic layer. The NH_4^+-N present in the flooded soil was readily oxidized to NO_3^--N (3.18 μg/cm^2/day) in the aerobic layer, the NO_3^--N formed diffused back (1.33 cm^2/day) into the anaerobic layer, where it underwent denitrification (15 μg/cm^3/day) into gaseous end products, such as N_2 and N_2O. This results in a concentration gradient of NH_4^+-N across the aerobic and anaerobic layers, which causes NH_4^+-N to diffuse upwards (0.216 cm^2/day) where it is subjected to nitrification-denitrification reactions. The experimental distributions of NH_4^+-N in the soil columns were not in close agreement with calculated distributions in the surface aerobic layer but were in close agreement in the anaerobic layer. It is possible that the rate constant (k) for NH_4^+-N oxidation varied considerably with depth in the aerobic layer and thus resulted in the disagreement. Applied labelled NH_4^+-N was rapidly lost from the soil system during a 4 month incubation period regardless of where NH_4^+-N was applied in the soil column. Nitrogen loss was lower when N was placed in the anaerobic zone than when placed at the aerobic surface. Addition of rice straw decreased loss of added N, but did not show any effect when N was applied to the overlying floodwater. Increasing the concentration of NH_4^+-N in the soil increased the N loss and also the thickness of the aerobic layer.

The effect of several cycles of varying length of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and loss of added and native N was investigated in a flooded

soil incubated for 128 days. Redox potential decreased rapidly when air was replaced with Ar for the short-time cycles, but decreased more slowly where the aerobic period was long enough to permit build up of NO_3^--N. Rate of decomposition of organic matter was faster in the treatments with a greater number of alternate aerobic and anaerobic periods. A maximum loss of 63.0% of applied labelled NH_4^+-N resulted from the shortest (2 and 2 day) aerobic and anaerobic incubation. Increasing the durations of the aerobic-anaerobic periods decreased the loss of N. Increasing the frequency of changing from aerobic to anaerobic conditions from 48 hour aerobic-48 hour anaerobic to 6 hour aerobic-6 hour anaerobic increased N loss. Nitrogen losses were somewhat less when the frequency was increased from 6 hour aerobic-6 hour anaerobic to 3 hour aerobic-3 hour anaerobic, but the loss of N again increased when the frequency was further increased to 1.5 hour aerobic-1.5 hour anaerobic. Nitrogen losses were due to alternate nitrification (during aerobic period) and denitrification (during anaerobic period) possibly coupled with chemical decomposition of NO_2^--N at the greater aerobic-anaerobic frequencies.

Introduction

Nitrogen reactions in flooded soils and sediments constitute an important part of the nitrogen cycle in nature. These reactions are mostly microbiological and involve both oxidation and reduction. The major reactions of soil nitrogen in a flooded soil involve organic N (due to immobilization or incorporation of inorganic N into biological tissue), NH_4^+-N (due to mineralization of organic N), NO_3^--N (due to nitrification of NH_4^+-N), N_2 and N_2O (gaseous products resulting from denitrification of NO_3^--N). Nitrogen is gained by a flooded soil mainly through added fertilizers, plant material and by nitrogen fixation. Nitrogen is lost from the flooded soil system through several processes, the major ones being denitrification, plant removal, volatilization of NH_3, leaching, NH_4^+-N fixation into crystal lattice, and erosion.

Nitrogen reactions in flooded soil are greatly influenced by the presence of O_2 in the atmosphere overlying floodwater. This O_2 will slowly diffuse through the floodwater and reach the soil surface, thereby causing the floodwater and the soil surface to be oxidized. The thickness of the aerobic soil layer (also called oxidized or nitrification zone) depends on the rate of O_2 movement through floodwater and soil and the rate of O_2 consumption by the soil. Generally the thickness of this layer varies from a few mm in soils of high

1

biological activity to 1 or 2 cm in soils of low biological activity. Underlying the aerobic soil layer is the anaerobic soil layer (also called reduced or denitrification zone), which is devoid of O_2.

The major inorganic form of N in flooded soil is NH_4^+-N. The NH_4^+-N present in the surface aerobic soil layer can be readily oxidized to NO_3^--N. The NO_3^--N thus formed during the nitrification reaction moves down into the anaerobic soil layer and undergoes denitrification (Figure 1). Several research workers, namely Pearsall (1950); Mitsui (1954); Patnaik (1965); Patrick and Tusneem (1972); Broadbent and Tusneem (1971); Manguit and Yoshida (1973); Patrick and DeLaune (1972); and Chen et al. (1972a) have reported this mechanism of N loss in flooded soils and sediments.

Rice plants have a unique feature of transporting atmospheric O_2 through the shoot and root system into the adjoining soil, thus creating an aerobic soil layer around the roots. Similar processes, as explained above, may presumably occur in the root zone having aerobic and anaerobic soil layers.

Flooded soils are subject to alternate flooding and draining during the crop period which establishes alternate anaerobic (reduced) and aerobic (oxidized) conditions in the soil. These conditions will enhance the loss of applied and native N through nitrification (when the soil is drained) and subsequent denitrification (when the soil is flooded). This situation can also be seen in upland soils, where temporary waterlogging can occur for short periods during rainy weather.

It is very important to have a quantitative measurement of the losses of applied NH_4^+-N that occur as a result of several biological

and physical processes in the flooded soil. These processes apparently control the availability of N to the rice plant.

The objectives of the investigations reported in this dissertation were: 1) to evaluate the major processes controlling N loss in a flooded soil, 2) to determine the extent of applied NH_4^+-N losses through nitrification-denitrification reactions as influenced by O_2 content overlying floodwater, 3) to determine the role of NH_4^+-N diffusion in N transport in the flooded soil system, and 4) to determine the effect of alternate aerobic (oxidized) and anaerobic (reduced) soil conditions on native and applied N loss. Tracer techniques involving the stable isotope of nitrogen, ^{15}N, were employed to follow the fate of applied N in the flooded soil.

Figure 1. A model describing the nitrification–denitrification reactions in flooded soils.

REVIEW OF LITERATURE

Nitrification-denitrification reactions in flooded soils and
sediments constitute an important part of the nitrogen cycle in na-
ture. These reactions are mostly microbiological and involve both
oxidation and reduction of nitrogen. Several review articles have
been published in the last decade on nitrogen transformations in
soils (Alexander, 1965; Broadbent and Clark, 1965; Campbell and Lees,
1967; Patrick and Mahapatra, 1968; Woldendorp, 1968; Delwiche, 1970;
Painter, 1970; Tusneem and Patrick, 1971; Ponnamperuma, 1972; Keeney,
1973; Payne, 1973; and Brejonik, 1975). Though these reviews discuss
the individual transformations in considerable detail, they do not
discuss the significance of the simultaneous occurrence of nitrifi-
cation-denitrification reaction. The agronomic and ecological signi-
ficance of the simultaneous occurrence of these processes in flooded
soils and sediments has become known only recently. This review will
be primarily concerned with the physical and chemical conditions in
flooded soils and sediments which influence these reactions. Figure
1 indicates the various transformations occurring simultaneously in
flooded soils and sediments.

A. Establishment of Aerobic and Anaerobic Soil

Layers as a Result of Flooding

Flooded soils and sediments are characterized by the absence of

5

O_2 compared to well drained soils. In a well drained soil, there is usually enough O_2 present in the soil atmosphere to act as an electron acceptor for microbial respiration. Upon flooding, the soil O_2 status is completely changed. In rice fields and shallow lakes, the dissolved O_2 content of the overlying floodwater remains relatively high due to: 1) a low density of O_2 consuming organisms, 2) photosynthetic O_2 production by algae, and 3) mixing of water by wind action and convection currents. Though the O_2 demand is low in the overlying floodwater, the demand is usually high in the underlying soil layer, especially in those soils having an appreciable organic energy source which supports a high level of microbial activity. Turner and Patrick (1968) could detect no O_2 in four soil suspensions within 36 hours of withdrawal of the O_2 supply. No O_2 was detected in submerged lake muds and flooded rice fields by several research workers (Armstrong and Boatman, 1967; Yunkervich et al. 1966; Kristensen and Enoch, 1964; Mortimer, 1941, 1942). Hutchinson (1957) estimated an O_2 consumption rate of 2×10^{-10} to 2×10^{-9} g cm^{-2} sec^{-1} in lake and ocean muds, whereas Howeler and Bouldin (1971) reported a value of 1×10^{-12} g cm^{-2} sec^{-1} in saturated soils. The low redox potentials reported by Hutchinson (1957) and Ponnamperuma (1965) for lake muds and rice soils, respectively, indicate the absence of O_2. Turner and Patrick (1968) found that the redox potential at which O_2 disappeared was in the range of +320 to +340 mv.

In shallow surface waters of flooded soils and lakes, dissolved O_2 moves slowly by diffusion and convection currents to the soil or sediment surface. The greater potential consumption of O_2 within the

flooded soil or sediment profile, compared to the renewal rate through
the floodwater results in the development of two distinctly different
soil layers: 1) an oxidized or aerobic surface layer where O_2 is pre-
sent, and 2) an underlying reduced or anaerobic layer in which no free
O_2 is present (Figure 2). This profile differentiation has been des-
cribed for lake and ocean muds and flooded soils by several research
workers (Pearsall, 1938; Pearsall and Mortimer, 1939; Mitsui, 1954;
Alberda, 1953; Pearsall, 1950; DeGee, 1950; Mortimer, 1942; Hayes and
Anthony, 1958; Friedman et al. 1968; Friedman and Gavish, 1970). Aer-
obic microorganisms function in the surface oxidized (aerobic) layer,
whereas in the reduced (anaerobic) layer, strict anaerobes and faculta-
tive anaerobes function.

The thickness of the aerobic surface soil or sediment layer is
determined by the net O_2 consumption rate in the soil and the O_2 re-
newal rate through the floodwater. The thickness of this layer gen-
erally varies from few millimeters in soils of high biological acti-
vity where there is greater demand for O_2 to 1 or 2 cm in soils of low
biological activity, where there is less demand for O_2 (Howeler and
Bouldin, 1971; Patrick and DeLaune, 1972; Engler and Patrick, 1974).
To calculate the thickness of the aerobic zone and the amount of O_2
that diffuses into the soil, six mathematical diffusion models were
developed by Bouldin (1968). Patrick and DeLaune (1972) character-
ized the oxidized (aerobic) and reduced (anaerobic) soil layers by mea-
suring the redox potential at different depths and the distribution of
reduced components such as sulfide and soluble iron and manganese.
The presence of a surface aerobic layer in lake and ocean muds is of
the utmost ecological importance because it acts as a sink for phos-

Figure 2. Development of aerobic (oxidized) soil layer.

phate and other plant nutrients (Hutchinson, 1957; Armstrong, 1965; Mortimer, 1971; McKee et al. 1970; Fitzgerald, 1970; Harter, 1968).

Oxygen reaching the soil or sediment surface is consumed in several ways:

(1) Oxygen is consumed by microbial respiration in the aerobic soil layer, where it is used as an electron acceptor.

(2) Oxygen is consumed by chemical oxidation of reduced iron and manganese which diffuse into the aerobic layer.

(3) Oxygen is consumed by the biological oxidation of NH_4^+-N. The organisms involved in the oxidation of NH_4^+-N to NO_3^--N are strictly aerobic.

Pearsall and Mortimer (1939) measured various redox components in the water above a lake bottom, in the aerobic surface mud layer, and in the underlying O_2-free mud, and reported the surface layer of mud contained the oxidized forms of iron, manganese, inorganic nitrogen, and sulfur, while the underlying mud contained reduced forms of these elements. Some of the O_2 consumption models describing the consumption of reduced iron were developed by Howeler and Bouldin (1971). Their studies showed that ferric iron was present in the aerobic (oxidized) layer and absent in the anaerobic (reduced) layer, while ferrous iron was absent in the aerobic layer and present in the reduced layer. Their studies also indicated that approximately 50% of the total available O_2 was used in oxidizing the water-soluble iron diffusing upwards from subsurface reduced zones, and the less mobile insoluble ferrous iron compounds encountered as the oxidized layer increased in depth. Patrick and DeLaune (1972) showed that the apparent

thickness of the aerobic layer was different when evaluated by the distribution of selected elemental components in the profile. The sulfide profile indicated the thickest aerobic zone, the manganese profile indicated the thinnest aerobic zone, and the iron profile was intermediate in thickness. No quantitative measurement is available to show the extent of O_2 consumption in oxidizing the reduced components of the soil.

The development of two distinct soil layers favor the simultaneous occurrence of nitrification and denitrification in a flooded soil or sediment. The thin surface aerobic (oxidized) layer favors the oxidation of NH_4^+-N to NO_3^--N, and the subsurface anaerobic (reduced) layer favors the reduction of NO_3^--N to N_2 and N_2O.

Rice soils are also subjected to alternate flooding and draining during the crop period, which results in alternating predominately anaerobic (reduced) and aerobic (oxidized) conditions in the soil with time. These conditions will enhance the loss of applied and native N through nitrification (when the soil is drained) and subsequent denitrification (when the soil is flooded). This situation can also develop in upland soils where temporary waterlogging can occur for short periods during rainy weather.

Rice and marsh plants have a unique feature of transporting atmospheric O_2 through the stem to the roots, and some of this O_2 subsequently diffuses from the root into the adjacent soil layer. This mechanism is undoubtedly one of the major factors enabling these plants to survive the anaerobic toxic environment of a flooded soil or sediment (Alberda, 1953; Aomine, 1962; Aimi, 1960; Rodriguez et al. 1965; Armstrong, 1964, 1967). The oxidation of the rhizosphere

appears to serve as a protective mechanism in preventing high concen-
tration of reduced substances from coming into contact with the root
surface. Besides oxidizing the reduced microzone around the root sur-
face, it will also enhance the development of a predominately aerobic
microflora in this zone (IRRI, 1964). The formation of these two dis-
tinct layers around a root surface (Figure 1) can also favor nitrification-
denitrification reactions.

B. Nitrogen Transformations in Flooded Soils

(i) Forms and Sources of Nitrogen

Nitrogen in flooded soils and sediments exists in two forms: 1)
inorganic nitrogen and 2) organic nitrogen. A large proportion of
soil nitrogen is present in organic combination with inorganic soil
nitrogen accounting for a small proportion of the total N. Inorganic
nitrogen is the form available for plant uptake. The different forms
of nitrogen present in the organic fraction of soils have been re-
cently reviewed by Bremner (1965a) and Tusneem and Patrick (1971).
The forms of nitrogen present in sediments was recently reviewed by
Keeney (1973).

Inorganic nitrogen is mainly present as NH_4^+ and NO_3^-. Nitrite may
also be present as an intermediate product of nitrification, denitri-
fication, and NO_3^- reduction. Ammonium nitrogen in flooded soils is
mainly derived through mineralization of organic nitrogen. Where O_2
is limiting in flooded soils and sediments, mineralization of organic
nitrogen does not proceed beyond the NH_4^+-N stage. The mineralization
and immobilization processes in waterlogged soils were reviewed in
great detail by Bartholomew (1965), Patrick and Mahapatra (1968), and

Tusneem and Patrick (1971). Another source of NH_4^+-N in flooded soils is through application of ammonium sulfate as a fertilizer to rice. This is the most widely used fertilizer around the world. The NO_3^--N in flooded soil is derived mainly through nitrification of NH_4^+-N. The special conditions under which NO_3^--N is formed in predominately reduced flooded soils is discussed in greater detail in the latter part of this report.

This review mainly focuses on the N transformations which are involved in converting NH_4^+-N into gaseous end products such as N_2 and N_2O. The reactions mainly include nitrification and denitrification.

(ii) Nitrification Reaction

Nitrification is defined as the biological oxidation of NH_4^+-N to NO_3^--N. In general, this process includes conversion of NH_4^+-N to NO_3^--N by autotrophic and heterotrophic organisms. Although there is evidence that some heterotrophic microbes are nitrifiers, it is generally believed that the contribution made by heterotrophs is negligible compared to autotrophs (Alexander, 1965; Keeney, 1973). Nitrification of NH_4^+ to NO_3^- involves two different groups of chemoautotrophic microorganisms. These nitrifiers derive energy from the oxidation of NH_4^+ and/or NO_2^-. These organisms utilize the O_2 reaching the soil surface in the oxidation of NH_4^+-N to NO_3^--N. There are two reactions involved in the conversion of NH_4^+-N to NO_3^--N, both of which are exothermic. These reactions are written as follows (Alexander, 1965; Delwiche, 1970):

Ammonium oxidation to nitrite:

$$NH_4^+ + 1\ 1/2O_2 \longrightarrow NO_2^- + 2H^+ + H_2O$$

The organisms involved belong to genus <u>Nitrosomonas</u>. The energy yield (ΔF) in the oxidation of NH_4^+ to NO_2^- was reported to have been -65.2 to -84.0 K cal per mole NH_4^+.

Nitrite oxidation to nitrate:

$$NO_2^- + 1/2O_2 \xrightarrow{\hspace{2cm}} NO_3$$

The organisms involved belong to genus <u>Nitrobacter</u>. The ΔF of the <u>Nitrobacter</u> reaction was reported to have been -17.5 to -20.0 K cal per mole (Gibbs and Schiff, 1960). These two dominant genera belong to the family Nitrobacteriaceae of the order Pseudonionadales (Alexander, 1965).

Combining these two reactions, we obtain:

$$NH_4^+ + 2O_2 \xrightarrow{\hspace{2cm}} NO_3^- + 2H^+ + H_2O$$

This reaction involves a N valence change from -3 to the +5 oxidation state, a span involving 8 electrons. It takes 2 moles of O_2 to oxidize each mole of NH_4^+ to NO_3. Though O_2 is essential, nitrification will occur readily down to about 0.3 ppm dissolved O_2. The actual limit is more dependent on the O_2 diffusion into the system rather than the O_2 level at a given time (Greenwood, 1962).

Nitrifiers are most active in a temperate range of 30 to 37 C (Frederick, 1956; Alexander, 1965), and the optimum pH is around neutrality. Under most soil conditions favorable to the overall nitrification process, the oxidation of NO_2^- proceeds at a faster rate than the oxidation of NH_4^+ and thus NO_2^- does not accumulate in a soil or sediment system. However, NO_2^- may accumulate to some extent under conditions of high pH or low temperature (Ulken, 1963; Alexander, 1965). High concentrations of NH_4^+ have also been found to inhibit the activity of <u>Nitrobacter</u>, particularly at high pH values, probably due

to production of free NH_3 (Aleem and Alexander, 1960; Erh et al. 1967).

Generally, fertilized soils have larger populations of nitrifiers compared to unfertilized soils (Alexander, 1965; Ardakani et al.1974a). In a field study, Ardakani et al.(1974b) estimated the population of Nitrobacter and Nitrosomonas micro-organisms at different depths in an aerated soil. Profile distribution of nitrifiers showed higher density near the soil surface where concentration of substrates and O_2 were always highest (Ardakani, 1973). Dunigan and DeLaune (1973) also observed a large number of nitrifiers in the surface layer of soil incubated under flooded conditions.

It is generally believed that the rate of nitrification follows a zero order. Lees and Quastel (1946) and Quastel and Schloefield (1949) reported that the rate of nitrification in the soil was independent of the NH_4^+-N concentrations in the soil solution. Later, several other workers showed the nitrification reaction to be independent of NH_4^+-N concentrations (Anderson, 1965; DeMarco et al. 1967; Wild et al. 1971; Kiff, 1972; Harkness, 1966). Ching-San-Huang et al. (1974) reviewed four reaction rate equations in a study conducted to mathematically describe the reaction rate. Their studies indicated the nitrification process is a zero-order reaction.

Nitrification has long been regarded as beneficial to crops grown in upland soils. However, recent realization that nitrification leads to significant losses of fertilizer N by leaching and denitrification and possibly to high NO_3^- levels in water supplies have caused researchers to revise their thinking on this topic (Keeney, 1970; Keeney and Gardner, 1970; Keeney, 1973). Nitrification is not bene-

ficial to rice grown in flooded soils. Nitrification occurs in the thin aerobic surface layer of a flooded soil, and the NO_3^- thus formed readily leaches down into the anaerobic layer and undergoes denitrification. The extent of losses caused by this sequential nitrification-denitrification process is discussed elsewhere in this section.

(iii) Denitrification Reaction

Denitrification reaction is defined as the biological reduction of NO_3^- or NO_2^--N to the gaseous end products such as molecular N_2 or N_2O. This definition excludes the chemo-denitrification (nonenzymatic) of NO_2^--N to N_2O and NO_2 and assimilatory reduction of NO_3^--N to NH_4^+-N. Recent investigations by Goering and Dugdale (1966), Kuznetsov (1968), Brezonik and Lee (1968), Keeney et al. (1971), and Engler and Patrick (1974) show denitrification to be an important process in removing N from lakes and floodwater. Under anaerobic or O_2-free conditions and in the presence of available organic substrate, the denitrifying organisms can use NO_3^- as an electron acceptor during respiration (Nicholas, 1963). This reaction can be carried out by many facultative anaerobes, mainly in the genera Pseudomonas, Achromobacter, Bacillus, Micrococcus, and Clostrodium (Alexander, 1961). When O_2 is available these organisms oxidize a carbohydrate substrate to CO_2 and H_2O:

$$C_6H_{12}O_6 \quad \text{------}\rightarrow \quad 6CO_2 + 6H_2O$$

Under O_2-free conditions, some micro-organisms oxidize a carbohydrate substrate to CO_2 and H_2O, using NO_3^- instead of O_2 as an electron acceptor and converting the NO_3^- to N_2 gas:

$$C_6H_{12}O_6 + 4NO_3 \quad \text{------}\rightarrow \quad 6CO_2 + 6H_2O + 2N_2$$

This reaction is irreversible in nature. The energy yield during

NO_3^- reduction is about 545 k cal per mole of glucose if the NO_3^- is reduced to N_2O and 570 k cal if NO_3^- is reduced all the way to elemental gaseous N_2 (Delwiche, 1970).

Cooper and Smith (1963), Cady and Bartholomew (1960), Nason (1962), Fewson and Nicholas (1961), and Chen et al. (1972a) have confirmed that the sequence $NO_3^- \longrightarrow NO_2^- \longrightarrow N_2O \longrightarrow N_2$ is operating in true biochemical denitrification.

The majority of NO_3^--N in waterlogged soils is from the nitrification of applied NH_4^+-N in the surface aerobic layer. The NO_3^- formed in the aerobic layer is constantly supplied to the anaerobic layer by diffusion in response to a concentration gradient and removed by denitrification reaction (Pearsall, 1950; Mitsui, 1954; Patrick and Gotoh, 1974).

Several factors are known to influence or regulate denitrification. These are namely, anaerobic conditions, organic matter status, pH, and temperature. Besides O_2-free conditions, organic matter is equally important because it is being used as an oxidizeable substrate to supply energy for anaerobes and also serves as a hydrogen donor in the denitrification process. Bremner and Shaw (1958) and Nommik (1956) reported that denitrification was greatly influenced by soil pH. High denitrification rates were observed in alkaline soils and reduced rates under neutral and acid conditions. Wijler and Delwiche (1954) observed that at pH values higher than 7, the denitrification reaction was more complete. The favorable temperature for the denitrification reaction is at 25 C and above. The transformation is still rapid at elevated temperatures and will proceed at about 60 to

65 C (Alexander, 1961). The effect of moisture content on denitrifica-
tion was shown by Jansson and Clark (1952), Nommik (1956), and Bremmer
and Shaw (1958). Pearsall and Mortimer (1939), and Patrick (1960)
showed that denitrification would occur when the redox potential of
the soil falls below 340 mv.

There is varied opinion on the kinetics of denitrification rates
in soils. There is agreement among several workers that denitrifica-
tion rate is independent of NO_3^- concentration (Broadbent and Clark,
1965). Patrick (1960) showed NO_3^- disappearance in soil suspension
followed zero-order kinetics. Stanford et al. (1975) described the
denitrification rate best by using a first-order rate equation rather
than a zero-order rate equation. The rates of denitrification in their
study were influenced by diffusion of NO_3^- from the floodwater to the
soil layer.

(iv) Nitrification-Denitrification Reaction

These two reactions are known to occur in flooded soils where
both aerobic and anaerobic conditions exist. The nitrification reac-
tion occurs in the surface aerobic layer, and denitrification reaction
occurs in the anaerobic soil layer. By combining these two reactions,
a balanced equation occurring in aerobic and anaerobic layers can be
written as:

$$24NH_4^+ + 48O_2 \longrightarrow 24NO_3^- + 24H_2O + 48H^+$$

$$24NO_3^- + 5C_6H_{12}O_6 + 24H^+ \longrightarrow 12N_2 + 30CO_2 + 42H_2O$$

$$24NH_4^+ + 5C_6H_{12}O_6 + 48O_2 \longrightarrow 12N_2 + 30CO_2 + 66H_2O + 24H^+$$

Four moles of O_2 is required to react with 2 moles of NH_4^+ to pro-

Figure.3. Schematic representation of nitrification-denitrification
reactions.

duce 1 mole of N_2 gas. The NO_3^--N appears as an intermediate product in the reaction and does not appear in the final reaction. This would be true where the surface aerobic layer is thin and little NO_3^- accumulates. A schematic representation of the pathways of these two reactions in terms of valence changes is shown in Figure 3.

(v) Effect of Aerobic and Anaerobic Soil Layers on Nitrogen Loss

The presence of aerobic and anaerobic soil layers profoundly affects the N economy of paddy soils, lake and ocean bottoms. As early as 1935, the importance of these aerobic and anaerobic layers was realized in studies of N transformations. Shioiri and Mitsui (1935) observed severe losses of applied NH_4^+-N in flooded soils during extended incubations. The losses were apparently too great to be accounted for by direct volatilization or by other possible mechanisms of N loss known at that time. Further experiments led them to believe that even under flooded conditions, NH_4^+ may be nitrified to NO_3^- if it is applied to the flooded soil surface. Nitrate thus formed moved down into the anaerobic zone by diffusion and was subsequently denitrified biologically and possibly chemically to gaseous end products such as N_2O and N_2 gas. Later, this process was confirmed by Pearsall (1950), Shioiri and Tanada (1954), and Mitsui (1954).

The importance of the nitrification-denitrification sequence in aerobic-anaerobic soil layers has been recognized by several research workers around the world. These reactions are undesirable in paddy soils, but may be ecologically beneficial in regulating excessive accumulation of nutrient forms of N in aquatic ecosystems, especially in wetland soils and sediment-water systems receiving large N inputs from

waste discharge sources. Vaccaro (1965) estimated that without de-
nitrification, N deposits in aquatic sediments would deplete the at-
mosphere N in 400 million years.

Studies conducted in paddy soils in different parts of the world
indicated that losses of N through nitrification and subsequent deni-
trification largely account for the low recovery of NH_4^+-N fertilizers
by rice (IAEA, 1966). Abichandani and Patnaik (1955) estimated these
losses to be 20 to 40%, while losses of 30 to 50% of applied N were
reported in Japan (Mitsui, 1954). Several other workers reported sim-
ilar losses through these mechanisms (Basak et al. 1957; Yamane, 1957;
Amer, 1960; Takijima, 1959; Subbiah and Bajoj, 1962). Patnaik (1965),
in a tracer study of N transformations in waterlogged soils, could
not account for 23 to 24% of applied N. Tracer investigations (IRRI,
Annual Report, 1965) on the fate of ammonium sulfate under submerged
conditions showed considerable losses of applied NH_4^+-N due to denitri-
fication after 4 weeks of incubation.

Tusneem and Patrick (1971), Broadbent and Tusneem (1971), and
Patrick and Tusneem (1972) using ^{15}N reported that substantial N
losses occurred in soils receiving N in a reduced form (ammonium sul-
fate) and maintained in a flooded condition throughout the experimen-
tal period. These studies demonstrate that nitrification and subse-
quent denitrification reactions were functioning simultaneously in a
flooded system and controlling N loss. Similar results were also re-
ported by Manguiat and Yoshida (1973) and Yoshida and Padre (1974).
In other recent studies, Patrick and DeLaune (1972), Patrick and Gotoh
(1974), Takai and Uehara (1973), showed that more NH_4^+-N was lost from

a flooded soil than is actually present in the aerobic soil layer at any one time. Apparently NH_4^+-N diffuses from the anaerobic soil layer to the aerobic soil layer where it undergoes nitrification and subsequent denitrification.

Application of fertilizer NH_4^+-N to flooded rice fields by deep placement has been shown to be superior to surface application (Mikkelsen and Finfrock, 1957; Patrick et al. 1967; Yanagisawa and Takahashi, 1964; IAEA, 1966; Broeshart and Middelboe, 1968; DeDatta et al. 1968; Merzari and Broeshart, 1968; DeDatta and Venkateshwarlu, 1968). Low recoveries of fertilizer N applied by surface placement compared to deep placement are believed to be caused primarily by the nitrification of NH_4^+-N in the aerobic layer and subsequent denitrification of NO_3^--N in the anaerobic layer. However, no significant differences in yield and N uptake of rice were observed when N was applied either by deep placement or by surface application of labelled fertilizer N (Reddy and Patrick, 1976a).

Recovery of applied fertilizer N in the soil-plant system under field conditions was determined by Patrick and Reddy (1976). Their studies indicated that when labelled ammonium sulfate was applied by deep placement, an apparent loss of 25% applied N occurred, whereas 49% of applied N was recovered in the plant, and 26% of applied N remained in the soil either in the roots or in the native soil organic matter. It was suggested that these N losses were due to NH_4^+-N diffusing from the anaerobic layer to the aerobic layer, where it undergoes nitrification and subsequent denitrification.

The importance of the nitrification-denitrification sequence has also been recognized by researchers working with environmental quality.

Chen et al. (1972b) showed that nitrification and subsequent denitrification functioned simultaneously in quiescent lake sediments incubated under air in the laboratory. Graetz et al. (1973) observed that during anoxic conditions, NH_4^+-N was released to the water at a relatively constant rate. Aeration effected rapid nitrification, and the NO_3^- thus formed in the overlying water decreased with time, possibly due to diffusion into the highly reduced sediment and subsequent denitrification. Kemp and Mudrochova (1972), working with Lake Ontario sediments, indicated tha N was returned to the atmosphere through nitrification-denitrification processes. Billen (1975) showed that nitrification of NH_4^+-N is the main source of increase in NO_3^- concentration in the upper layers of the sediment. When this NO_3^- reached below the 3-cm layer, it disappeared by denitrification. Brujewicz and Zaitseva (1972), working with ocean sediments, reported the distribution of NH_4^+-N with depth. Their results indicate that the concentration of NH_4^+-N was low in the overlying water and surface sediment layer, while high NH_4^+-N levels were found in the lower sediment layers. These results suggested that NH_4^+-N is probably disappearing through nitrification reactions. Recently, Curtis et al. (1975) estimated that 80% of the oxidation of NH_4^+-N occurred in the sediments only.

Nitrogen losses in flooded soils and sediment can be greatly influenced by the presence of plants. The magnitude of N loss can be reduced by the uptake of inorganic N present in soil solution. The N losses can be enhanced by the presence of the aerobic layer around the root zone through sequential mechanism of NH_4^+ diffusion from the anaerobic (reduced) layer into the aerobic (oxidized) layer around the

root, nitrification of NH_4^+-N to NO_3^--N in the aerobic layer around the root, diffusion of NO_3^--N from the aerobic layer to the anaerobic layer, and finally denitrification of NO_3^--N to gaseous end products. Woldendorp (1968) suggested that plant roots can accelerate denitrification in the rhizosphere by taking up O_2 and by secreting organic substances which can serve as H^+ donors in the denitrification process. Workers at IRRI (1964) assessed the relative abundances of certain micro-organisms occurring in the rhizosphere. Their data indicated the presence of nitrifying bacteria around the root zone. At present, no information is available on the activity of nitrifying organisms in the oxidized rhizosphere of predominately reduced soils. It is speculated, however, that the high root density of rice plants in flooded soil can increase the total area of the aerobic zone and create a favorable condition for nitrification to occur.

(vi)   Effect of Alternate Aerobic and Anaerobic Conditions on

        Nitrogen Loss

Inorganic N transformations are greatly influenced by alternate aerobic and anaerobic conditions. Severe N loss has been shown to occur in soils subjected to alternate draining (aerobic) and flooding (anaerobic) (Wijler and Delwiche, 1954; Russell, 1961; Patrick and Wyatt, 1964; MacRae, Ancajas and Salandanan, 1968). Organic N is converted to NH_4^+-N in both aerobic and anaerobic soils (though the nitrogen mineralization rate is reported to be greater in aerobic soils). The NH_4^+-N thus formed is oxidized to NO_3^--N (under aerobic conditions only) and the resulting NO_3^--N denitrified (under anaerobic conditions only). Loss of N by sequential nitrification and denitrification is

especially high in soils planted to lowland rice, where water management practices sometimes require frequent draining and reflooding.

Russell and Richards (1917) were among the first to recognize the importance of alternate aerobic and anaerobic conditions in determining loss of N. Their studies showed large losses of N from farmyard manure under alternate wetting and drying conditions, but little loss when the material was maintained under either aerobic or anaerobic conditions. Wijler and Delwiche (1954) noted that alternating aerobic and anaerobic conditions should result in greater total N loss from the soil than would be found under continuous anaerobic conditions. Greenland (1962) pointed out that nitrification-denitrification could proceed simultaneously, possibly due to a micromosaic of aerobic and anaerobic spots in wet soil. Patrick and Wyatt (1964) observed large losses of N (up to 20% of total N or 200 ppm) as a result of repeated cycles of flooding and drying to field moisture. Tusneem and Patrick (1971) showed that NH_4^+-N was highly unstable under alternate flooded and moist conditions of several weeks duration, since only trace amounts of applied labelled NH_4^+-N was recovered at the end of a 120-day incubation period. Alternate aerobic and anaerobic conditions are not useful agronomically, whereas ecologically, these reactions could be of considerable significance in removing NH_4^+-N from wastewater.

THEORETICAL CONSIDERATIONS

In a flooded soil NH_4^+-N is stable in the anaerobic soil layer but may be nitrified in the aerobic soil layer. If the entire flooded soil profile is initially at a uniform concentration of NH_4^+-N, nitrification in the aerobic soil layer causes a concentration gradient to develop between the aerobic-anaerobic layers. Therefore, NH_4^+-N diffuses from the anaerobic soil layer to the aerobic soil layer. As nitrification proceeds, the increase in concentration of NO_3^--N in the aerobic layer results in a diffusive flux of NO_3^--N from the aerobic soil layer to the anaerobic soil layer. The NO_3^--N in the anaerobic soil layer may then be denitrified and eventually lost from the system as N_2 gas.

Laboratory experiments were conducted in order that the measured concentration distribution of NH_4^+-N and loss of NH_4^+-N from a flooded soil could be compared to the theoretical concentration distribution and loss of NH_4^+-N. The geometry of the diffusion problem is shown in Figure 4. The second order differential equations describing concentration of NH_4^+-N as a function of soil depth and time and the initial and boundary conditions are as follows.

Aerobic soil layer:

$$\partial c/\partial t = D\partial^2 c/\partial x^2 - k, \quad -a < x < 0 \qquad [1]$$

$$c(x,0) = c_o, \quad -a < x < 0 \qquad [2]$$

$$D\ \partial c(-a,t)/\partial x = 0, \quad t < 0 \qquad [3]$$

25

Figure 4. The geometry of the diffusion problem.

Anaerobic soil layer:

$$\partial C^*/\partial t = D\ \partial^2 C^*/\partial x^2,\quad 0 < x < +\infty \qquad [4]$$

$$C^*(x,0) = C_o,\quad 0 < x < +\infty \qquad [5]$$

$$\lim_{x \to \infty} C^*(x,t) = C_o,\quad t > 0 \qquad [6]$$

Aerobic-anaerobic soil layer interface:

$$C(0^-,t) = C^*(0^+,t),\quad t > 0 \qquad [7]$$

$$D\ \partial C(0^-,t)/\partial x = D\ \partial C^*(0^+,t)/\partial x,\quad t > 0 \qquad [8]$$

where

C = concentration of NH_4^+-N in aerobic layer, $\mu g/cm^3$ of soil

C^* = concentration of NH_4^+-N in anaerobic layer, $\mu g/cm^3$ of soil

D = apparent diffusion coefficient of NH_4^+-N in soil, cm^2/day; since the soil is saturated, D should remain constant and be the same value in both the aerobic and anaerobic layers.

k = rate constant of zero-order reaction for nitrification in aerobic layer, $\mu g/cm^3/day$.

C_o = initial concentration of NH_4^+-N in soil column assumed to be constant, $\mu g/cm^3$ of soil.

x = space coordinate, cm

t = time, days

a = depth of aerobic layer, cm

Equations [1] and [4] are the same except [1] accounts for nitrification in the aerobic soil layer (and is in effect a sink for NH_4^+-N) while equation [4] contains no sink term since NH_4^+-N is stable in the anaerobic layer. Equations [2] and [5] state that the concentration of NH_4^+-N in the soil column is uniform and of constant value C_o at

time equal to zero. Equation [3] states that there will be no flux

of NH_4^+-N from the aerobic soil layer to the overlying floodwater; the

depth of the floodwater is assumed to be infinitely small. Equation

[6] states that the concentration of NH_4^+-N at the lower end of the

soil column will not change during the duration of the experiment.

Equation [7] insures that the concentration functions at the inter-

face of the aerobic-anaerobic layer are equal and continuous. Equa-

tion [8] insures that the diffusive flux of NH_4^+-N out of the anaer-

obic soil layer equals the diffusive flux into the aerobic soil layer.

The solutions of equations [1] and [4] subject to the initial

and boundary conditions, (equations [2], [3], [5], [6], [7], and [8])

are as follows:

Aerobic soil layer, $-a < x < 0$

$$C(x,t) = C_o - kt + \frac{k}{2} \{ [t + (-x)^2/(2D)] \; \text{erfc} \; [-x/2(Dt)^{1/2}]$$

$$- (-x)(t/\pi D)^{1/2} \exp[-(-x)^2/4Dt]$$

$$+ [t + (x + 2a)^2/(2D)] \; \text{erfc}[(x + 2a)/2(Dt)^{1/2}]$$

$$- (x + 2a)(t/\pi D)^{1/2} \exp [-(x + 2a)^2/4Dt] \} \qquad [9]$$

Anaerobic soil layer, $0 < x < +\infty$

$$C^*(x,t) = C_o - \frac{k}{2} \{ (t + x^2/2D) \; \text{erfc} \; [x/2(Dt)^{1/2}]$$

$$- x(t/\pi D)^{1/2} \exp[-x^2/4Dt]$$

$$- [t + (x + 2a)^2/(2D)] \; \text{erfc} \; [(x + 2a)/(2(Dt)^{1/2})]$$

$$+ (x + 2a)(t/\pi D)^{1/2} \exp [-(x + 2a)^2/(4Dt)] \} \qquad [10]$$

The quantity, $F(t)$ of NH_4^+-N which has diffused from the anaerobic

layer into the aerobic soil layer at any time, t, can be found by the

use of equation [11].

$$F(t) = D\int_o^t [\partial C^*(0,t)/\partial x] \; dt$$

$$= (2/3)k \{ [(3/2)(at) + (a^3/D)] \; \text{erfc}[a/(Dt)^{1/2}]$$

$$+ (D/\pi)^{1/2} t^{3/2} [1 - \exp [-(a)^2/(Dt)]]$$

$$- a^2 [t/(\pi D)]^{1/2} \exp [-(a)^2/(Dt)]\}$$ [11]

The erfc (Z) used above is standard notation for the complementary error function; e.g., erfc $Z = 1 - (2/\pi^{1/2}) \int_0^z e^{-y^2} dy$. It should be noted that equation [11] is independent of C_o, the initial concentration of NH_4^+-N in the soil. It should be pointed out that equations [9] and [10] cannot be used indiscriminately since equation [11] assumes that nitrification is a zero-order reaction; for example, for sufficiently long times, small C_o values and/or large k values, negative values for C or C* can be calculated from equations [9] and [10]. If this happens, one must realize that NH_4^+-N cannot be nitrified if there is no NH_4^+-N present.

The solutions, equations [9] and [10], were found with the use of the Laplace transformation. The procedure of using the Laplace transformation can be found in Operational Mathematics textbooks such as Churchill (1958) or in Carslaw and Jaeger (1959). The derivation of equations [9], [10] and [11] will be furnished to the reader upon request.

MATERIALS AND METHODS

The soil used in the present studies was Crowley silt loam,

(Typic Albaqualfs) obtained from Rice Experiment Station, Crowley,

Louisiana. The soil was air dried, ground to pass through a 10 mesh

sieve, mixed thoroughly and stored in a tightly sealed container. For

each independent study a subsample of the soil was taken and ground to

pass 40 mesh sieve. Certain physical and chemical properties of the

soil are shown in Table 1. The N source used in the experiments was

either ammonium sulfate or potassium nitrate and was thoroughly mixed

with the soil. This was accomplished by mixing the N source with a

small amount of soil in a porcelain mortar and then mixing with a larg-

er amount of soil to obtain desired concentration of NH_4^+-N or NO_3^--N.

Later the soil containing N source was kept on a roller mixer for sev-

eral hours to insure uniform mixing with the soil. In the experiments

dealing with tracer study, ^{15}N-tagged nitrogen was introduced into the

soil system.

Description of Experiments

Experiment I

Evaluation of Major Processes Controlling
Nitrogen Loss in Flooded Soil

Four major processes namely, NH_4^+-N diffusion, nitrification,

NO_3^--N diffusion and denitrification were evaluated in independent

experiments.

30

Table 1. Certain soil properties of Crowley silt loam used in the
present experiments.

Total carbon	0.70%
Total nitrogen	0.08%
NH_4^+-N	8.0 µg/g
NO_3^--N	18.0 µg/g
Soil pH (1:1 soil water ratio)	5.6
Cation exchange capacity	9.4 meq/100 g soil
Clay	10.8%
Silt	70.7%
Sand	9.5%

Ammonium Diffusion:

This experiment was designed to show the movement of NH_4^+-N from a zone of high concentration (anaerobic soil layer) to a zone of low concentration (aerobic soil layer) in a flooded soil. The soil was incubated under two different atmospheric conditions. One incubation was carried out under an anaerobic atmosphere and the other under an aerobic atmosphere overlying the floodwater. The NH_4^+-N concentration gradient was established by adding 200 µg N/g of soil as NH_4^+-N in the lower zone of the soil column (Zone A as shown in Figure 5) and no N added in the upper zone of the soil column (Zone B). The soil was incubated in PVC cores of 4.8 cm inside diameter and 10 cm length. A mixture of paraffin wax and petroleum jelly (2:1 ratio) was poured into the bottom of the cylinder to provide a 1 cm thick base, which was later used as support to push the soil from the PVC cylinder for sectioning. Fifty grams of soil containing 200 µg N/g of soil was then added to the cylinder with enough distilled water (20 ml to wet the soil. The soil was allowed to settle for half an hour and an additional 20 ml of distilled water was added to the core and then 50 g of untreated soil (with no added N) was added. The total length of the soil column was 4.8 cm. Finally 60 ml distilled water was added to provide 2.5 cm of overlying floodwater. The bulk density of the soil was 1.145 g/cm^3 and the moisture content was 47% by weight and 54% by volume. The cylinders were then placed in a larger flask that was purged with argon. The anaerobic atmosphere prevented the formation of an aerobic soil layer. The soils were incubated for 1, 2, 4, 8, 15, 30 and 60 days in darkness at 28 C. At the end of each incubation period the soil columns were sliced into 2 mm sections and vertical

Figure 5. Simple apparatus used to study ammonium diffusion in flooded soil.

distribution of NH_4^+-N was measured in the soil column. The D values were calculated from 1- and 2-day incubation periods. The sectioning of the soil columns was carried out by the method described by Reddy and Patrick (1976b).

The second study was designed to show the effect of NH_4^+-N removal by nitrification in the aerobic soil layer on NH_4^+-N movement from the underlying anaerobic zone. An aerobic soil layer was developed by incubating the soil column under an aerobic (21% O_2) atmosphere. Soil columns were prepared as described in the earlier experiment and incubated for 2, 4, 8, 15, 30 and 60 days. At the end of each incubation period the soil columns were sliced into 2 mm sections and analyzed for NH_4^+-N concentration.

The geometry of the diffusion problem followed in the calculation for NH_4^+-N diffusion was the same as that described by Phillips and Brown (1964).

$$D = \pi h^2 F^2 / t$$

where:

D = apparent diffusion coefficient (cm^2/day)

h = length of soil column (cm)

F = ratio of $\dfrac{Q_1 + Q_2}{Q_1}$

Q_1 = total quantity of ions diffused

Q_2 = total quantity of ions remaining in the soil

t = time (days)

Rate of Nitrification Reaction:

An experiment was carried out to measure the rate and order of the nitrification reaction in the aerobic soil layer. Fifty grams of

Crowley silt loam having 200 μg N per g of soil as NH_4^+-N was weighed into a wide mouth bottle (in duplicate) to provide a layer of soil 1 cm thick. The soil layer was completely saturated and a thin layer of floodwater was maintained. The sample bottles were then exposed to air (21% O_2) for a period of 0, 2, 4, 8, 15, 30 and 60 days. A thin layer of soil was used in order to obtain oxygenated conditions throughout the soil.

In another study, 500 g of soil was stirred continuously with an equal amount of water while air was bubbled through the suspension for 6 days to maintain aerobic conditions. The soil suspension was then treated with 100 μg NH_4^+-N per g of soil. A known amount of soil suspension was transferred to several wide mouth bottles to obtain 1 cm thick oxygenated soil layer. The samples were incubated for a period of 0, 4, 8, 15, 30 and 60 days and then analyzed for NH_4^+-N remaining in the soil.

Rate of nitrification reaction was calculated based on zero order reaction.

$$\frac{d[NH_4^+]}{dt} = k$$

where:

  k = rate constant for ammonium N disappearances μg/cm³/day

  t = time (days)

Nitrate Diffusion:

This experiment was designed to show the movement of NO_3^--N from the overlying floodwater (where a large supply of NO_3^--N was maintained) into the underlying soil layer. One-hundred and fifty grams Crowley

silt loam with an equal amount of water containing 0.1% formaldehyde solution, was weighed into PVC cores (in duplicate) and incubated under anaerobic atmosphere for 5 days. Formaldehyde was used to supress the denitrifying organisms. The overlying floodwater was treated with 300 μg NO_3^--N per ml of floodwater as KNO_3 and the cores were further incubated for a period of 24 hours. At the end of incubation, the NO_3^--N remaining in the floodwater and the NO_3^--N diffused into the underlying soil layer was determined after sectioning the soil core into 2 mm sections.

The geometry of the diffusion problem followed for NO_3^--N moving from floodwater into the soil column was the same as that described by Phillips (1976, Personal Communication).

$$D = F^2/4C_o^2 t$$

where:

D = diffusion coefficient of NO_3^--N, cm^2/day

F = total quantity of NO_3^--N moved into the soil column

C_o = initial concentration of NO_3^--N at the soil surface

t = time (days)

Rate of Denitrification Reaction:

This study was designed to measure the rate of the denitirfication reaction in the anaerobic soil layer. Fifty grams of Crowley silt loam and 25 ml distilled water receiving different amounts of NO_3^--N (100 and 200 μg N/g of soil) as KNO_3 was weighed into 6 oz. bottles. The bottles were then fitted with serum caps and purged several times with N_2 gas to displace O_2. The bottles were incubated for a period of 0, 1, 2, 4, 6, 8 and 12 days in the dark at 28 C and were

analyzed for NO_3^--N at the end of each incubation.

Rate of denitrification was calculated based on the first order rate reaction.

$$\ln \frac{[NO_3]_t}{[NO_3]_o} = k\ [NO_3]_o$$

where:

k = rate constant for NO_3^--N disappearance µg/day

$[NO_3]_t$ = nitrate concentration at any given time

$[NO_3]_o$ = initial NO_3^- concentration

t = time (days)

Distribution of Labelled NH_4^+-N in a Flooded Soil Column:

This experiment was designed to measure the distribution of applied $^{15}NH_4^+$ in the aerobic and anaerobic soil layers of a flooded soil incubated under air. Four PVC cylinders (10.5 cm diameter and 15 cm long) with a paraffin wax base to facilitate extruding and sectioning were used. Five-hundred grams of soil containing 200 µg labelled NH_4^+-N per g of soil were weighed into PVC cores and incubated under saturated conditions in an argon atmosphere for a period of 1 week. Later the flooded soil was exposed to an aerobic atmosphere (21% O_2) for 60 days. At the end of incubation the cores were sliced into 2 mm sections and analyzed for labelled N. The redox profile of the core was also measured (Patrick and DeLaune, 1972).

Experiment II

Gaseous Losses of Applied Ammonium Through Nitrification-Denitrification Reactions, As Influenced by Oxygen Content of the Overlying Floodwater

This experiment was designed to determine the role of O_2 in

Figure 6. Incubation flask used in the experiment to measure the gaseous end products.

determining nitrogen loss through the nitrification-denitrification reaction.

Fifty grams of soil containing labelled N with an equal amount of water was weighed into a special type flasks (Figure 6), provided with ground glass joints and sampling outlet to attach to the mass spectrometer. Argon and O_2 were mixed at different proportions based on partial pressures to obtain a total pressure of 1 atm. in the flask. The percent O_2 used in the study was 0, 5, 12, 26 and 50 and the remaining gas in the flask besides O_2 was argon. Duplicate flasks for each treatment were perfectly sealed and incubated for total period of 100 days at 28 C in a dark incubation room. A trap of ascarite crystals was provided in the incubation flask to absorb any CO_2 produced during incubation period in the flask. The development of aerobic soil layer was measured manually by recording the color developed by ferric iron. Similarly another set of incubation flasks in duplicate were set up with 30% O_2 and incubated for a period of 0, 7, 15, 30, 60 and 100 days.

At the end of incubation period duplicate flasks were shaken thoroughly to drive out any gases trapped in the soil and attached to a mass spectrometer with a special type of attachment provided to the gas sampling system of the flask. The total pressure in the flask was measured by attaching the flask to a manometer. The total N_2 and N_2O produced during the incubation period were directly obtained from a spectrum recorded by the instrument and was directly related to standard gases spectrum recorded by same instrument. The procedure used in the calculation was discussed in detail by Van Cleemput et al. (1976). The peaks of N_2O were corrected for any contribution from

CO_2 based on m/e 22. The fragmentation of N_2O, which also contributes to the m/e 28, 29 and 30 were also taken into consideration. After making all corrections for secondary peaks the isotopic ratios were calculated for 28/29 and 44/45 from which the atom fraction of ^{15}N excess was calculated. The gaseous calculations made in this experiment were similar to that of reported by Cady and Bartholemew (1960). All N fractions studied were computed on nitrogen basis. After obtaining the gas samples, the soil in the flasks was quantitatively transferred to another bottle and analyzed for labelled N, in NH_4^+-N, NO_3^--N and organic N fractions.

The O_2 consumption in the flask during the incubation period was calculated by difference from the mass spectrometric data. The total O_2 present at the beginning and at the end of experiment was calculated from the spectrum obtained in the mass spectrometry based on m/e 32 which was later related directly to the standard gaseous spectrum obtained for O_2. The O_2 consumed during nitrification-denitrification reaction was also calculated.

Experiment III

Ammonium Diffusion as a Factor in Nitrogen Loss From Flooded Soil

This study was carried out to determine the net transport of NH_4^+-N from the anaerobic layer to aerobic layer, and to also compare the experimental value obtained for NH_4^+-N disappearance resulting from nitrification-denitrification reactions with those calculated from theoretical equations developed to describe the NH_4^+-N loss from flooded soil.

Plexiglass columns (24 cm long and 4.5 cm diameter) were used to prepare soil columns such that all initial and boundary conditions imposed by the theoretical model were satisfied. The plexiglass columns were provided with a 1-cm thick base of a paraffin-petroleum gel (2:1 ratio). Soil containing 200 μg NH_4^+-N/g of soil was added to the columns with enough water to completely saturate the soil. The soil in the columns had a bulk density of 1.145 g/cm^3 and an NH_4^+-N content of 229 $\mu g/cm^3$. The atmosphere above the soil in the columns was completely displaced with argon and incubated for a period of one week to insure uniform anaerobic conditions throughout the soil column. The soil columns were then covered with a thin layer of floodwater and incubated in the dark for periods of 7, 15, 30, 60, 90, and 120 days at 28 C under the atmosphere level of O_2 (21%). At the end of each incubation period the soil columns were horizontally sliced into 2-mm sections without freezing the soil (Reddy and Patrick, 1976b). Each sectioned sample was rapidly dried at 40 C and analyzed for NH_4^+-N by direct steam distillation.

Experiment IV

Effect of Placement and Concentration of Applied Ammonium N on Nitrogen Loss from Flooded Soil

Two independent experiments, one to determine the effect of placement of NH_4^+-N on N loss and the other on the concentration of applied NH_4^+-N on its loss, were conducted. The description of experiment is given as follows.

Experiment 1: This experiment was designed to measure the loss of N from a flooded soil as influenced by placement of NH_4^+-N. The following treatments were used:

(A) NH_4^+-N applied uniformly throughout a 4-cm soil column

(B) NH_4^+-N applied to upper 1 cm of the soil column

(C) NH_4^+-N applied to lower 1 cm of the soil column

(D) NH_4^+-N applied to the overlying floodwater

To follow the fate of applied NH_4^+-N, labelled N was used at a rate of 100 μg/g of soil. To measure the effect of organic matter on N loss, this experiment was carried out with and without added rice straw. One-hundred grams of soil (oven dry basis) was used for each treatment which gave a 4-cm column. An overlying floodwater depth of 2.0 cm was maintained throughout the incubation period. In treatment A, NH_4^+-N was uniformly mixed with the soil. In treatment B and C, NH_4^+-N was applied to only one fourth portion of the soil column at a rate of 400 μg N/g of soil (which was equivalent to 100 μg N/g of soil on whole soil col-umn basis). Finely ground rice straw at 0.2% concentration was uni-formly mixed with the soil. Duplicate samples were incubated for per-iods of 0, 7, 15, 30, 60, 90, and 120 days at 30 C and analyzed for to-tal and labelled N (in both organic and inorganic fractions). The thickness of the aerobic soil layer was measured for treatment A by slowly driving a platinum electrode through the soil column and record-ing redox potential (Patrick and DeLaune, 1972).

Experiment 2: This study was carried out to find the extent of N loss at varying rates of NH_4^+-N application to a flooded soil and the effect of NH_4^+-N concentration on the development of the aerobic soil layer. Duplicate 100 g samples of soil containing 0, 100, 200, and 400 μg N/g of soil as $(NH_4)_2SO_4$ were weighed into wide mouth bottles and incubated at 30 C under flooding for periods of 0, 7, 15, 30, 60, 90, and 120 days. Another set was incubated in PVC cylinders for a

period of 90 days to study the vertical distribution of applied NH_4^+-N. At the end of the incubation period the soil columns were sliced horizontally into 2-mm sections and analyzed for both NH_4^+-N and NO_3^--N content. The redox profile of the soil column was also measured.

Experiment V

Effect of Long Term Alternate Aerobic and Anaerobic Condition on Redox Potential, Organic Matter Decomposition and Nitrigen Loss in Flooded Soil

This study was designed to show the effect of alternating aerobic and anaerobic periods on loss of both native and added N and on soil organic matter decomposition. Changes in the redox potential of the soil under these alternate aerobic and anaerobic conditions were also measured. The various treatments used are shown in Table 4.

Duplicate flasks for each treatment were set up as shown in Figure 7. One hundred and fifty grams of soil was weighed into each of the two incubation flasks along with 300 ml distilled water to give a soil to water ratio of 1:2 and then incubated at 30 C under constant stirring with a magnetic stirrer for a period of 128 days. An energy source of 0.5 per cent finely ground rice straw (48 percent carbon) and 100 μg/g N in the form of $(NH_4)_2SO_4$ containing 10.1458 atom per cent excess ^{15}N were thoroughly mixed with the soil. Aerobic and anaerobic conditions were accomplished by bubbling either CO_2-free air or Ar through the incubation flasks. A platinum electrode was permanently inserted through a rubber stopper to measure the redox potential of the soil suspension. The flasks were connected by means of glass and rubber tubing for continuous flow of CO_2-free air or Ar for creating aerobic or anaerobic conditions. The CO_2 evolved during microbial

Figure 7. Diagram of the apparatus used for studying the effect of long term aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil.

respiration was trapped in a 0.5 $\underline{N}$ KOH solution.

Redox potential measurements were made by connecting the platinum electrode to a pH meter, using a saturated calomel half cell as reference electrode (Patrick and Wyatt, 1964). A complete cell was formed by inserting a salt bridge (glass tube) through a serum cap in the rubber stopper of the flask. Redox potential measurements were made daily during the incubation period of 128 days. The pH of soil suspension at the end of 128 day incubation period was also measured. The CO_2 produced during decomposition of organic matter was analyzed according to the procedure given by Stotzky (1965). Total carbon remaining in the soil at the end of incubation period was analyzed by the dry combustion method (Allison, Bollen and Moodie, 1965).

Experiment VI

Effect of Short Term Alternate Aerobic and Anaerobic Conditions on Redox Potential and Nitrogen Loss

This study was designed to measure the effect of even more frequent aerobic-anaerobic changes on N loss. An energy source of 0.5% finely ground rice straw (48% carbon) and 100 µg N/g of soil as $(NH_4)_2SO_4$ or KNO_3 containing 10.146 or 10.077 atom % excess ^{15}N, respectively, were thoroughly mixed with the soil.

Duplicate flasks for each treatment were set up as described in Experiment V with few modifications. Alternate aerobic and anaerobic conditions were established by passing either air (21% O_2) or Ar (O_2 free) through a three-way solenoid valve attached to a timer as shown in Figure 8. The timers were set at desired intervals (as given in Table 5) and air and Ar were supplied automatically for a desired period. There was a rapid turnover of gas in the flask with a displace-

1. Solenoid valve (three-way)
2. Platinum electrode
3. Salt bridge
4. Calomel half-cell
5. 0.1N H_2SO_4
6. Water trap
7. One-way valve
8. Magnetic bar

Figure 8. Diagram of the apparatus used to study the effect of short
term aerobic and anaerobic conditions on redox potential and nitrogen
loss in a flooded soil.

ment time of 2 minutes. Redox potential measurements were made using a platinum electrode and a saturated calomel half cell. The redox potential was detected with a millivolt meter connected to a strip chart recorder.

The study was carried out for two different incubation periods. In Experiment VIa the total incubation period was 128 days with the treatments varying from 6 hours aerobic and 6 hours anaerobic to 48 hours aerobic and 48 hours anaerobic. In Experiment VIb the total incubation period was 64 days with the treatments varying from 1.5 hours aerobic and 1.5 hours anaerobic to 6 hours aerobic and 6 hours anaerobic. All treatments are listed in Table 1. In Experiment VIa the samples were analyzed at the end of the incubation period for NH_4^+-N, NO_3^--N, and organic N. In Experiment VIb samples were removed during the incubation period at 0, 4, 8, 16, 32, and 64 days, and analysis was carried out for NH_4^+-N, NO_3^--N, NO_2^--N, and organic N. Nitrate and NO_2^--N were analyzed by the phenoldisulfonic acid and the Griess-Ilosvay methods, respectively. Ammonium N was analyzed using steam distillation and nesslerization and organic-N by the modified Kjeldhal method (Bremner, 1965a). Labelled N was determined in both organic and inorganic fractions using a Dupont Model 21-614 isotope ratio mass spectrometer (Bremner, 1965b).

Analytical Methods:

    1. Determination of Inorganic Nitrogen

        a. Extraction

Inorganic nitrogen $(NH_4^+ + NO_3^-)$ was extracted from the soil by shaking the soil samples for one hour with 2 $\underline{N}$ KCl solution,

adjusted to pH 2.5 and were then filtered using a Buchner funnel. During the filteration, the soils were washed with excess KCl solution. After extraction the soil was dried in the air draft oven at 40 C and stored for organic N analysis. The filterates were used for NH_4^+-N and NO_3^--N analysis.

b. Determination of Ammonium N

The NH_4^+-N in the extract was determined by distilling over (using macro Kjeldhal distillation apparatus) after adding excess MgO. The distillates were collected in 0.1 N H_2SO_4. The samples containing a high NH_4^+-N concentration were titrated with standard NaOH (0.1 N) using a mixed indicator (described in detail, Bremner, 1965b). In the samples containing less than 2 mg N, a small aliquot from the distillates was taken and Nessler's reaction was carried out. The intensity of color was measured at 425 mμ on a Fisher colorimeter. The distillates were further prepared for ^{15}N analysis.

c. Determination of Nitrate N

After distillation of NH_4^+-N, the samples were cooled to room temperature and distilled over after adding Deverda's alloy to reduce NO_3^--N to NH_4^+-N. The reduced NH_4^+-N was further determined as described above. The solutions were further prepared for ^{15}N analysis.

d. Determination of Ammonium and Nitrate N in the Smaller Soil Samples (obtained from 2-mm sections of soil columns)

The soil columns, after sectioning were immediately transferred to a sample dish and approximately 5 ml of 2 N KCl (pH 2.5) was added. Each sample was quantitatively transferred into a micro Kjeldhal flask by carefully washing the soil into the flask with 2 N KCl. Then the

sample was distilled over after adding MgO, using a steam distillation apparatus (Bremner, 1965) into 10 ml of 0.1 $\underline{N}$ H_2SO_4. Later the samples were further diluted to a known volume, and nessler's reaction was carried out. The intensity of color was measured at a wavelength of 425 mμ using a Fisher colorimeter. The NO_3^--N in the sectioned samples was analyzed by following phenolodisulfonic acid method, as described in detail by Bremner (1965b).

2. Determination of Organic Nitrogen

After the extraction of inorganic nitrogen, the soil was oven-dried at 40 C and ground to pass a 60-mesh sieve. A soil sample equivalent to 5.00 g (oven dry basis) was analyzed in duplicate for organic nitrogen. The soil sample was weighed into a Kjeldahl flask (650 ml), and 10 ml of deionized distilled water was added. The soil was then allowed to stand for 20 minutes. The flask was swirled carefully, and 10 g of digestion mixture (10 parts K_2SO_4, 1 part $FeSO_4$, 1/2 part $CuSO_4$) was added. Then 20 ml of concentration H_2SO_4 was added, and the contents were mixed by swirling the flask. Digestion then commenced and continued for at least 3 hours after the solution had cleared. The digested material, diluted to 250 ml with deionized distilled water, was allowed to cool to room temperature, and was distilled after adding 75 ml of 50% NaOH and a few pieces of zinc metal. The NH_3 released by distillation was collected in standard 0.1 $\underline{N}$ H_2SO_4 and determined by titration with standard 0.1 $\underline{N}$ NaOH. The titrated solution was further prepared for ^{15}N analysis.

3. Determination of Labelled Nitrogen

The titrated samples or samples which were distilled over con-

taining nitrogen in the form of ammonium sulfate, were evaporated to a concentrated form so that the concentration of the final solution was approximately 1 mg $\underline{N}$ per ml. The concentrated samples were transferred to glass vials and stored in the refrigerator until analyzed.

The ^{15}N analyses were carried out using Dupont Model 21-614 mass spectrometer having an isotope ratio attachment. The procedure followed is essentially the same as described by Bremner (1965c), with few modifications.

a. Preparation of Sodium Hypobromite Solution

Fifty grams of NaOH was dissolved in 400 ml deionized distilled water while cooling. Cooling was accomplished by keeping the bottle in a container having ice cubes. After cooling the solution to room temperature, 15 ml of liquid bromine was added, a few milliliters at a time with cooling while stirring. After all bromine was added, the solution was diluted to a total of 500 ml by adding 85 ml deionized distilled water. The solution was stored in the refrigerator. When this reagent solution became six months old, the reagent was discarded and a fresh reagent was made. The reagent was stored at least a week in the refrigerator before using for the first time.

b. Conversion of Ammonium N to Nitrogen Gas

The conversion of NH_4^+-N to nitrogen gas for mass spectrometer analysis was carried out by treatment of 1 ml of sample (in some cases up to 3 ml of sample to keep the concentration of NH_4^+-N to 1 mg) with 3 ml of alkaline sodium hypobromite solution in the total absence of air (vacuum down to 1 μ pressure). The N_2 gas evolved is shown by the following reaction:

$$2NH_3 + 3NaOBr = 3NaBr + 3H_2O + N_2$$

This reaction was accomplished in a modified Rittenberg Y-tube designed to fit the mass spectrometer gas inlet system. About three glass beads on each side of the Y-tube were added to avoid bumping during vacuum. The N_2 gas generated through this reaction was passed through a U-tube immersed in liquid nitrogen. The purified nitrogen gas was allowed into the instrument at a constant pressure of 100 μ. The intensity of m/e 28 and 29 peaks were measured at an ionizing current of 50 μ amps. For each sample three spectrum were recorded and then peak heights were measured and further calculations were carried out.

c. Calculation of Results

The mass spectrometer measured the ratio of the intensities of the currents produced by two isotopic ion beams. In the case of nitrogen, the measured ratio (R) is that of the ion currents corresponding to mass 28 and 29:

$$R = \frac{[^{14}N\ ^{14}N]}{[^{14}N\ ^{15}N]} \qquad (1)$$

Following ions are formed upon ionization of N_2 gas, namely $[^{14}N\ ^{14}N]$, $[^{14}N\ ^{15}N]$, $[^{14}N\ ^{15}N]$, and $[^{15}N\ ^{15}N]$. The distribution of these ions can be shown by the equation:

$$^{14}N\ ^{14}N + {}^{15}N\ ^{15}N = {}^{14}N\ ^{15}N + {}^{14}N\ ^{15}N \qquad (2)$$

Theory and experiment give a value of 4.00 for the equilibrium constant of this reaction at room temperature.

The definition of atom percent ^{15}N is given as:

$$= \frac{\text{Number }^{15}N\text{ atoms x 100}}{\text{Number }^{14}N\text{ atoms + Number }^{15}N\text{ atoms}}$$

$$= \frac{[^{14}N\ ^{15}N] + 2[^{15}N\ ^{15}N]}{2[^{14}N\ ^{14}N] + 2[^{14}N\ ^{15}N] + 2[^{15}N\ ^{15}N]} \times 100 \qquad (3)$$

Therefore:

$$\frac{[^{14}N\ ^{15}N]^2}{[^{14}N\ ^{14}N]\ [^{15}N\ ^{15}N]} = 4 \qquad (4)$$

The final equations were obtained upon combining the above equations 1, 2, and 3

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

Further calculations for labelled N recovered were made from the following equation:

$$x = \frac{(N \cdot Ar) - (N \cdot An) - (Bn \cdot An)^*}{Ao - An}$$

where:

x = labelled nitrogen

An = atom percent ^{15}N in natural standard

Ao = atom percent ^{15}N originally added

Ar = atom percent ^{15}N recovered at the end of experiment

N = total N (whichever fraction under study)

Bn = nitrogen due to blank (contributed through reagents)

*This parameter can be eliminated from the equation, if the contribution of nitrogen due to blank is very low.

RESULTS AND DISCUSSION

Experiment I

Evaluation of Major Processes Controlling Nitrogen Loss in Flooded Soil.[1]

Ammonium Diffusion:

Movement of NH_4^+-N from a soil zone of high concentration to a soil zone of low concentration was measured in a flooded soil under two different atmospheric conditions (with and without O_2) above the floodwater. The distribution of NH_4^+-N in Zone A (high NH_4^+-N concentration) and Zone B (low NH_4^+-N concentration) are presented in Figures 9 and 10 for several incubation periods. The diffusion coefficient (D) for the NH_4^+-N moving in a saturated Crowley silt loam was estimated from the distribution curve obtained for 1 and 2 days because these two incubations satisfied the initial and boundary conditions imposed by the equation derived by Phillips and Brown (1964). The average estimated D value for NH_4^+-N moving in a saturated silt loam was 2.51×10^{-6} cm^2/sec (0.216 cm^2/day). The D value reported by Berner (1974) for NH_4^+-N moving in sediments containing 70% water was 3.5×10^{-6} cm^2/sec. These D values reported for wet soils and sediments are generally higher than the D values of 0.4 to 3.0×10^{-7} cm^2/sec obtained for well drained soils by Clarke and Barley (1968). High soil moisture content is known to influence the ion diffusion

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[1]This section was presented at the 67th Annual Meeting of the American Society of Agronomy, during August 25-28, 1975 at Knoxville, Tennessee.

53

Anaerobic atmosphere overlying floodwater

Figure 9 – continued.

Anaerobic atmosphere overlying floodwater

Figure 9. Movement of ammonium N from the zones of high ammonium N
concentrations to the zones of low ammonium N concentrations in
the soil columns incubated under anaerobic atmosphere above floodwater.

Aerobic atmosphere overlying floodwater

Figure 10. (Continued).

Aerobic atmosphere overlying floodwater

15 days	30 days	60 days

Depth — mm

NH_4^+ Concentration — µg/g

Figure 10. Movement of ammonium N from the zones of high ammonium N
concentrations to the zones of low ammonium N concentrations in
the soil columns incubated under aerobic atmosphere above floodwater.

(Phillips and Brown, 1964, 1966; Rowell, Martin and Nye, 1967; Olsen and Kemper, 1968; Clarke and Barley, 1968).

The net movement of NH_4^+-N from Zone A to Zone B was calculated from the distribution curves obtained for several incubation periods (Figure 11). The net transport of NH_4^+-N increased for the whole period of incubation although the rate of transport was reduced with time. Initially the concentration gradient was higher between two zones but was reduced as incubation continued. After 60-day incubation about half of the NH_4^+-N applied in Zone A was transported into Zone B. The diffusion process of NH_4^+-N ion in flooded anaerobic soil may be influenced by other metal cations present. The major cations occupying most of the exchange complex are Fe^{+2} and Mn^{+2}, which exist in the reduced state. The abundance of these cations on the exchange complex will displace NH_4^+-N to the soil solution and enhance the transport of NH_4^+-N in flooded soil. The NH_4^+-N diffused into the floodwater was not taken into consideration in the present study. However, analyses indicated very little NH_4^+-N in the floodwater.

In another study where the atmosphere above the floodwater was aerobic (21% O_2) a similar trend of NH_4^+-N movement from Zone A to Zone B was observed up to a period of 15 days. After this period, with the development of an aerobic surface layer, the accumulated NH_4^+-N in the surface soil layers disappeared rapidly as a result of the nitrification reaction. The absence of an aerobic soil layer in the set where the atmosphere overlying the floodwater was anaerobic resulted in a high concentration of NH_4^+-N in the surface layer (Zone B), where as the presence of an aerobic soil layer where an aerobic atmosphere overlying the water was used, showed very low concentration of NH_4^+-N

Figure 11. Total ammonium N transported from the zones of high ammonium
.N concentrations to the low ammonium N concentrations at various
incubation periods.

in the surface soil layer (Zone B). This was due to the fact that NH_4^+-N in the aerobic soil layer was nitrified to NO_3^--N, because nitrifying bacteria function only in the presence of O_2 (Alexander, 1965).

Another study was carried out with longer soil columns for a 17-day incubation period, such that the soil column would be completely reduced in both Zones A and B. Care was taken that the distribution of NH_4^+-N in the soil column satisfied the initial and boundary conditions imposed by the equation (Phillips and Brown, 1964). The estimated D value was 4.1×10^{-6} cm^2 sec^{-1} for the NH_4^+-N ion moving in a saturated silt loam under anaerobic conditions. The value obtained here is twofold as compared to the value obtained for 1- and 2-day incubation period. The increased D value shows an indication that the reduced cations may enhance the NH_4^+-N movement in the flooded soil.

Rate of Nitrification Reaction:

Nitrification is the next important process that controls the N loss in a flooded soil. This is a biological reaction and involved organisms which are strictly aerobic and function only in the presence of O_2. The rate of NH_4^+-N is completely dependent on the number of nitrifying bacteria and abundance of O_2 in the surface soil layer. The optimum percentage of O_2 for rapid NO_3^--N production in soil is similar to that found in air (Grechin and Ch'eng, 1960) and low or unnaturally high partial pressures of O_2 suppress the activity of organisms. So the nitrification rates of well aerated soils cannot be compared with the nitrification rates of aerobic soil layer of a flooded soil. The aerobic soil layer was completely saturated with water and O_2 and the supply of O_2 decreases with increase in depth. Excessive

Figure 12. Rate of ammonium N disappearance in the aerobic soil layer (rate of nitrification reaction).

moisture content affects the rate of nitrification in soils (Alexander, 1965), but no data is available on nitrification rates in soil saturated with both water and O_2. The nitrification process followed a zero order reaction (Figure 12) indicating that NH_4^+-N oxidation was independent of NH_4^+-N concentration in the aerobic soil layer. The rate constant (k) calculated for zero order reaction of NH_4^+-N oxidation to NO_3^--N was 3.18 $\mu g/cm^3/day$ for Crowley silt loam soil under saturated conditions. It is essential, however, to determine the nitrification rates in the aerobic soil layer at different depths, since the O_2 supply decreases as depth increases.

Nitrate Diffusion:

Nitrate thus formed during nitrification process in the aerobic soil layer moves down into the anaerobic soil layer. The rate of NO_3^--N movement in the flooded soil is of utmost importance in controlling N loss. The NO_3^--N movement from the overlying floodwater into the underlying soil was measured and presented in Figure 13. The estimated D value was 1.5×10^{-5} cm^2/sec for Crowley silt loam. The high D value for NO_3^--N is naturally expected because NO_3^--N being anion does not get adsorbed on exchange complex and tends to move down much faster. The saturated conditions of the soil column enhance the NO_3^--N movement from overlying floodwater to underlying soil. Romkins and Bruce (1964) measured D value of 1.33×10^{-5} cm^2/sec for NO_3^--N in silt having 42.7% moisture (by volume). Whereas Clarke and Barley (1968) reported that NO_3^--N moving in a well drained soil has D values ranging from 0.5 to 5.0×10^{-6} cm^2/sec, which is very low as compared to the values obtained in the present experiments and by others. In compari-

Figure 13. Rate of nitrate movement from the overlying floodwater to the underlying soil layer of a flooded soil.

son with the D values obtained for NH_4^+-N, the NO_3^--N ions move much faster in a flooded soil. Rapid movement of NO_3^--N from overlying floodwater and aerobic soil layer into the underlying anaerobic soil layer decreases the accumulation of NO_3^--N in the aerobic soil layer.

Rate of Denitrification Reaction:

Nitrate reduction (denitrification reaction) is the process that occurs mostly in the absence of O_2, and in flooded soils it occurs in the anaerobic soil layer which is free of O_2. Under flooded conditions NO_3^--N is derived upon oxidation of NH_4^+-N in the surface aerobic soil layer. For NO_3^--N to be reduced into gaseous end products, it needs to move down from aerobic soil layer or from overlying floodwater to anaerobic soil layer. The rate of NO_3^--N reduction is dependent on three major processes which occur prior to NO_3^--N reduction; they are, namely, NH_4^+-N diffusion, NH_4^+-N oxidation, and NO_3^--N diffusion. The amount of energy source present in the anaerobic soil layer also affects the NO_3^--N reduction process. The rate of NO_3^--N disappearance is shown in Figure 14 and 15 for two initial concentrations of NO_3^--N. The rate of NO_3^--N reduction followed first order reaction, with a rate constant (k) of 0.075 and 0.31 day^{-1} for 200 and 100 μg of NO_3^--N/g of soil respectively. Since the soil was not shaken continuously during incubation, the diffusion of NO_3^--N influenced the order of reaction. The NO_3^--N concentration approached zero in 12 days in the treatment where 100 μg NO_3-N/g of soil was applied, whereas about 100 ug NO_3^--N/g of soil disappeared in 12 days when 200 μg NO_3^--N/g of soil was applied at the beginning of the experiment.

Figure 14. Rate of nitrate disappearance in the anaerobic soil layer of the flooded soil.

Figure 15. Rate of nitrate reduction shown as: $\ln \dfrac{[NO_3^--N]_t}{[NO_3^--N]_o}$ vs. time.

Distribution of Applied ^{15}N in Flooded Soil Core:

The distribution of applied $^{15}NH_4^+$-N in a flooded soil core after 60-day incubation period is presented in Figure 16. The presence of both the aerobic and anaerobic soil layers are shown in Figure 10, as shown by redox profile. Initially the $^{15}NH_4^+$-N was present uniformly throughout the soil core. At the end of incubation the labelled N was recovered in several fractions, namely organic fraction (13.4%), NH_4^+-N fraction (48.6%), NO_3^--N fraction (2.2%), and the remaining was accounted for loss, which was calculated by difference (Figure 16). Major part of applied $^{15}NH_4^+$-N remained as $^{15}NH_4^+$-N fraction only. The $^{15}NH_4^+$-N concentration approached zero at the aerobic soil layer and water interface and gradually the concentration of $^{15}NH_4^+$-N increased with increase in depth (up to 48 mm) to a maximum concentration of 140 μg N/g of soil. Similarly the distribution of $^{15}NO_3^-$-N derived from applied $^{15}NH_4^+$-N was also presented in Figure 17. Only 2.2 percent of applied $^{15}NH_4^+$-N was recovered as $^{15}NO_3^-$-N in the aerobic soil layer and the remaining was lost. The concentration of NO_3^--N approached zero at the aerobic and anaerobic soil layer interface, which was established at +300 mV as shown in redox profile (Figure 17). This clearly indicated that part of NO_3^--N was lost through denitrification in the aerobic soil layer, before it reached the anaerobic soil layer. Turner and Patrick (1968) have shown that the disappearance of O_2 at +340 mV and simultaneous occurrence of denitrification in the system already containing O_2.

All the NH_4^+-N which disappeared did not show up completely either in organic fraction or NO_3^--N fraction. The unaccounted N was 35.8%. These losses were too high to account for direct voltalization of NH_3

Figure 16. Distribution of labelled N in the aerobic and anaerobic soil layers of a flooded soil.

or due to NH_4^+-N fixation. The Crowley silt loam does not possess high pH to voltalize NH_3 or has high fixing capacity to remove large portions of NH_4^+-N into the crystal lattice. The disappearance of NH_4^+-N occurred all through the profile. The NH_4^+-N disappearance in the aerobic soil layer can be very well explained as the nitrification of NH_4^+-N to NO_3^--N in the aerobic soil layer and the NO_3^--N formed in the aerobic layer readily leached down into the anaerobic soil layer, where it is denitrified. The continuous disappearance of NH_4^+-N through nitrification process in the aerobic soil layer establishes a concentration gradient with respect to NH_4^+-N concentration through the aerobic and anaerobic soil layers. At this gradient level the NH_4^+-N from the anaerobic soil layer readily diffuses into the aerobic soil layer where it is further converted into NO_3^--N. The NO_3^--N accumulated will readily leach down into anaerobic soil layer, where it will be denitrified into gaseous end products such as N_2 and N_2O. These chain processes continue to function in natural flooded soil or sediment system, through long periods, as long as the above conditions are satisfied. The appearance of labelled NO_3^--N in the aerobic soil layer indicate the intermediate step of NH_4^+-N to N_2 or N_2O, through the above process explained.

High rate of NO_3^--N diffusion ($D = 1.5 \times 10^{-5}$ cm^2/sec) and high rate of NO_3^--N consumption ($k = 0.075$ and 0.31/day for 200 and 100 μg NO_3^--N per g of soil), show that these two processes are not limiting steps in controlling applied NH_4^+-N loss. Slow rate of NH_4^+-N diffusion ($D = 2.5 \times 10^{-6}$/cm^2/sec) and slow rate of nitrification ($k = 3.18$ μg/ cm^3/ day) indicate that these two processes are the limiting factors in controlling applied NH_4^+-N loss. If the nitrification reaction does

Figure 17. Distribution of labelled ammonium and labelled nitrate N (derived from applied ammonium N) in the aerobic and anaerobic soil layers.

not occur in the surface aerobic soil layer, the concentration gradient of NH_4^+-N will not be established across the aerobic and anaerobic soil layers. Nitrification reaction is independent of the NH_4^+-N concentration, so it does not depend on the amount of NH_4^+-N diffused from the anaerobic soil layer. This shows that nitrification reaction in the aerobic soil layer functions independently, whereas NH_4^+-N diffusion is dependent on rate of nitrification. Another important process controlling N loss is the O_2 diffusion from the overlying floodwater which aids in the development of the aerobic soil layer. When there is greater demand for O_2 in the floodwater and the surface soil layer, the aerobic soil layer will be very thin because of high biological activity (Engler and Patrick, 1974). This restricts the thickness of nitrification zone (aerobic soil layer) and further influences the NH_4^+-N disappearance. When there is lesser demand for O_2, the thickness of aerobic layer will be thicker and increase N loss (Patrick and Gotoh, 1974).

Experiment II

Gaseous Loss of Applied Ammonium N Through Nitrification-Denitrification Reaction, as Influenced by Oxygen of the Overlying Floodwater.[2]

Results of this study dealing with the effect of O_2 on NH_4^+-N conversion to NO_3^--N and then to N_2 gas are shown in Figure 19. The ammonium content of the system decreased with time with a buildup of N_2

Figure 18. Thickness of aerobic (oxidized) layer as influenced by the oxygen content overlying floodwater.

Figure 19. The conversion of ^{15}N-labeled ammonium to elemental nitrogen in a flooded soil. The distribution of labeled ammonium among various nitrogen fractions following incubation under 30% oxygen.

gas evident after two weeks. No N_2O was present until the last sampling at 100 days. Nitrate was present after 30 days, but did not accumulate. The most striking result of this experiment was the rapid conversion of NH_4^+-N to N_2 gas after 30 days. This length of time was approximately the same as that required for the development of a pronounced aerobic surface layer in the flooded soil. A small amount of labelled NH_4^+-N was incorporated into the organic fraction at the beginning of incubation and did not change appreciably in concentration during the 100 day period.

The O_2 content of the atmosphere over the flooded soil during the 100 day incubation period had a marked effect on the stability of NH_4^+-N. Where no O_2 was present almost all of the added labelled NH_4^+-N remained in the NH_4^+ form except for the small amount incorporated into the organic fraction. Where O_2 was present, however, at least part of the NH_4^+ was converted to N_2 gas, with the amount increasing as the O_2 content increased up to 26%. A higher O_2 content resulted in a thicker aerobic layer and slightly more NO_3^--N, but no additional N_2 gas (Figures 18 and 20).

These experiments utilizing tracer nitrogen demonstrate that NH_4^+-N in a flooded soil is converted to NO_3^--N and then to elemental N_2 gas. Measurements of the thickness of the aerobic surface layer and of the amount of labelled N_2 gas produced showed that approximately twice as much NH_4^+-N was converted to NO_3^--N and elemental nitrogen as was originally present in the aerobic layer.

These experiments have shown that appreciable denitrification will occur in flooded soils if both O_2 from the atmosphere and NH_4^+-N from the flooded soil are available (Patrick and Gotoh, 1974).

Figure 20. The conversion of ^{15}N-labeled ammonium to elemental nitrogen in a flooded soil. The effect of oxygen content on conversion of labeled ammonium to various nitrogen fractions.

Figure 21. Processes involved in the sequential conversion of organic nitrogen to elemental nitrogen in flooded soils and sediments.

Although the nitrogen converted from NO_3^- to gaseous forms in flooded systems is derived from NH_4^+-N oxidized to NO_3^--N in the aerobic layer, the amount of nitrogen gas usually greatly exceeds the amounts of NH_4^+-N and NO_3^--N present in the aerobic surface layer at any one time. Ammonium movement from the underlying anaerobic layer to the surface aerobic layer is apparently necessary to account for the large N losses that occur in flooded systems. Removal of NH_4^+-N by nitrification in the aerobic layer creates a concentration gradient which causes NH_4^+-N in the underlying anaerobic layer to diffuse upward to the aerobic layer where it undergoes nitrification. Nitrate formed by this process readily diffuses down toward the nitrate-free anaerobic layer where it is denitrified to N_2 gas by serving as an electron acceptor in the oxidation of organic matter. These processes are illustrated in Figure 21. As indicated in the equation, NO_3^--N is an intermediate in the overall pathway between NH_4^+-N and elemental N_2.

Oxygen consumption in the flooded soil is shown in Figure 22. Most of the O_2 consumed initially was probably used for oxidation of organic carbon by heterotrophs, but after several days a considerable portion was also being used for nitrification. Some of this O_2 was present as NO_3^--N, while some had been converted from NO_3^--N to CO_2 in the denitrification reaction (see equation in Figure 21). Increasing the concentration of O_2 over the flooded soil increased the thickness of the aerobic layer and increased the amount of O_2 consumed. Nitrification and organic carbon oxidation accounted for approximately equal amounts of O_2.

Figure 22. Total oxygen consumption, oxygen converted to nitrate, and oxygen converted sequentially to nitrate and then to carbon dioxide (see equation in Figure 21) as affected by time of incubation and oxygen concentration.

The results of this study show that NH_4^+-N in an anaerobic soil or sediment exposed to atmospheric O_2 undergoes sequential nitrification and denitrification. The source of the NH_4^+-N reacting with atmospheric O_2 in the aerobic layer consists of the NH_4^+-N initially present in the aerobic surface layer of soil or sediment plus an approximately equal amount diffusing to the aerobic layer from the underlying anaerobic layer. It is likely that a significant part of the NH_4^+-N present in flooded soils and shallow water sediments follows this pathway and makes a major contribution to the elemental nitrogen being returned to the atmosphere.

Experiment III

Role of Ammonium Diffusion on N Loss From Flooded Soil.[3]

Development of Aerobic Soil Layer:

The thickness of aerobic soil layer was measured in the anaerobic soil columns after exposure to the aerobic atmosphere overlying floodwater for different periods of incubation. This was done based on the reddish-brown color developed by ferric iron in the soil, the thickness of this colored layer being directly related to the thickness of the aerobic soil layer (Pearsall and Mortimer, 1939; Howeler and Bouldin, 1971). The difference in color of this layer and the dark grey color developed by the anaerobic soil layer was easily detected. During the first week of incubation, a very thin aerobic soil layer developed on the surface of the anaerobic soil. After 15 days the aerobic soil

Figure 23 . Relationship between the thickness of the oxidized (aerobic) layer measured by the presence of nitrate and the oxidized (aerobic) layer measured by the ferric iron color development.

layer increased to a depth of 0.5 cm and further increased to 1.25, 1.5, 1.6 and 2.0 cm after 30, 60, 90, and 120 days incubation respectively. The thickness of the aerobic soil layer was probably determined by the supply of O_2 and the readily decomposable amount of organic matter (Engler and Patrick, 1974; Patrick and DeLaune, 1972). The thickness of the aerobic soil layer as measured by the ferric iron color development was in close agreement with the NO_3^--N distribution in the soil columns (Figure 23).

Distribution of NH_4^+-N and NO_3^--N in the Aerobic and Anaerobic Soil Layers:

The distribution of NH_4^+-N in the aerobic and anaerobic soil layers is presented in Figures 24a, 24b and 24c. The distribution of extractable NH_4^+-N was fairly uniform throughout the soil column after 7 days, except for a slight disappearance at the surface. After 15 days NH_4^+-N steadily decreased in both aerobic and anaerobic soil layers. The disappearance was more striking in the columns incubated for 30 to 120 days. The possible mechanisms involved in the disappearance of NH_4^+-N are explained as follows. The NH_4^+-N present in the surface aerobic soil layer was readily oxidized to NO_3^--N. This is a biological reaction, which requires O_2 (Alexander, 1965). Volz et al. (1975) showed NH_4^+-N oxidizers decreased with depth in the soil columns. Immediately following nitrification in the aerobic soil layer a concentration gradient of NH_4^+-N was established across the aerobic and anaerobic soil layers. The NH_4^+-N present in the anaerobic soil layer diffused across the aerobic soil layer and was further subjected to nitrification and subsequent denitrification reactions. The amount of

Figure 24 a. The distribution of applied ammonium N in the aerobic and anaerobic soil layers of flooded soil columns.

Figure 24 b. The distribution of applied ammonium N in the aerobic and anaerobic soil layers of flooded soil columns.

Figure 24 c. The distribution of applied ammonium N in the aerobic and anaerobic soil layers of flooded soil columns.

NO_3^--N in the aerobic soil layer was low at any given time and decreased with depth as shown in Figure 25, indicating denitrification of NO_3^--N. The aerobic soil layer acts as a sink for NH_4^+-N diffusing from the anaerobic soil layer, whereas the anaerobic soil layer acts as a sink for the NO_3^--N diffusing from the aerobic soil layer (Figure 4). The presence of an aerobic surface layer in a flooded soil, lake and ocean bottom is of great importance, as it also acts as a sink for several other nutrients such as phosphate, iron and manganese (Armstrong, 1965; Mortimer, 1971; Fitzgerald, 1970; McKee et al. 1970), and the presence of an underlying anaerobic soil layer aids in the removal of NO_3^--N from the overlying floodwater (Engler and Patrick, 1974).

The distribution of NH_4^+-N in both the aerobic and anaerobic soil layers was also expressed as the change in NH_4^+-N concentration. Change in NH_4^+-N concentration was calculated from the equation:

$$C = C_o - C_t$$

where

C_o = concentration of NH_4^+-N in lower soil layer from which no NH_4^+-N diffusion has occurred.

C_t = concentration of NH_4^+-N in the profile at particular incubation period.

C = change in NH_4^+-N concentration.

The C_o chosen in this equation includes any mineralization and immobilization processes occurring in the soil column, but it assumes that immobilization and mineralization processes were constant throughout the soil column (Tusneem and Patrick, 1971). This assumption was essentially valid, since as can be seen in Figure 26 little or no decrease in NH_4^+-N concentration occurred at depths greater than 12 cm.

Figure 25. The distribution of nitrate N in the aerobic and anaerobic soil layers of flooded soil columns after different incubation periods.

Figure 26. The decrease in ammonium N concentration in the aerobic and anaerobic soil layers of flooded soil columns at different incubation periods.

Rapid decrease in NH_4^+-N concentration occurred in the surface soil layer (first 2 mm) and the remaining part of the soil column showed no decrease in concentration of NH_4^+-N in the anaerobic soil layer probably due to mineralization of organic N. After 15 days incubation there was a marked decrease in the NH_4^+-N concentration, especially in the surface aerobic soil layer. The change in the NH_4^+-N concentration was very rapid in the surface layers of the aerobic soil layer compared to the lower part of the aerobic soil layer. The decrease in concentration of NH_4^+-N in the anaerobic layer can be directly related to the diffusion process. The NH_4^+-N concentration continued to decrease as indicated by the levels found after the incubation periods of 30, 60, 90, and 120 days. Rapid disappearance of NH_4^+-N occurred in the aerobic soil layer during the 90-day and 120-day incubation period; the decrease in NH_4^+-N concentration was high as 230 $\mu g/cm^3$ in the surface soil layers.

The diffusion coefficients (D) for NH_4^+-N and NO_3^--N, and rate of nitrification in the aerobic layer were experimentally measured in independent experiments (unpublished results) for Crowley silt loam. The D value for NH_4^+-N and NO_3^--N was found to be 0.216 and 1.33 cm^2/day, respectively, whereas rate of nitrification was found to follow zero order kinetics with a rate constant of 3.18 $\mu g/cm^3/day$. The denitrification rates are generally high for Crowley silt loam (Patrick, 1960). A combination of rapid NO_3^--N diffusion and high NO_3^--N reduction rate was the probable factor for low accumulation of NO_3^--N in the aerobic layer (Figure 25). Thus the sequence of events regulating the loss of applied NH_4^+-N from flooded soil include NH_4^+-N diffusion,

Conc. NH_4^+-N—$\mu g\ cm^{-3}$

Figure 27. Comparison of the experimental and theoretical distributions of ammonium N in the aerobic and anaerobic soil layers of flooded soil columns after 90-day incubation period.

nitrification, NO_3^--N diffusion, and denitrification.

Distribution of NH_4^+-N as predicted by the theoretical equations (Page 25) did not agree well with the experimental distributions in the aerobic soil layer for the 90-day incubation period, but did agree more closely in the anaerobic soil layer (Figure 27). The experimental values showed much greater depletion of NH_4^+-N in the aerobic soil layer as compared to the theoretical values. A reasonable explanation for the disagreement of the experimental and theoretical distributions of NH_4^+-N concentration in the aerobic soil layer is that the k value (oxidation of NH_4^+-N to NO_3^--N) decreased with depth in the aerobic soil layer; in other words, the oxidation of NH_4^+-N to NO_3^--N decreases as redox potential decreases or as O_2 concentration decreases. However, this explanation has not been validated. Another reason for the disagreement of the two distribution curves is that the depth of the aerobic soil layer was assumed to be constant (1.5 cm) in the theoretical distributions while in the experimental columns the depth of the aerobic soil layer increased as a linear function of $t^{1/2}$. The influence of the moving boundary of the aerobic soil layer on NH_4^+-N loss from flooded soils and sediments is presently being investigated in this laboratory.

The total NH_4^+-N disappearing from the soil column was calculated for the experimental distributions by the following equation (Figure 28):

$$N_t = s \int_{-a}^{+\infty} (C_o - C_t) \, dx$$

where

N_t = total NH_4^+-N disappearance

C_o = initial NH_4^+-N concentration

Figure 28. Typical distribution curve used in the calculation of ammonium N loss in the aerobic and anaerobic soil layers of flooded soil columns.

C_t = concentration of NH_4^+-N at the end of incubation period

S = cross sectional area of the soil column

x = depth (cm)

$\int_{-a}^{+\infty} (C_o - C_t)\, dx$ represents the amount of NH_4^+-N disappearance and was calculated graphically.

The values obtained are presented in Figure 29 on g of N/m^2 basis. Similarly the NH_4^+-N disappearing in the aerobic soil layer was also calculated using the same equation, where $\int_{-a}^{0} (C_o - C_t)\, dx$ represents NH_4^+-N disappearance in the aerobic soil layer only. The net loss of NH_4^+-N due to diffusion was calculated from $N_d = N_t - N_o$, where, N_d = NH_4^+-N disappearing due to diffusion only, N_t = total NH_4^+-N disappearing from the soil column, and N_o = NH_4^+-N disappearing in the aerobic soil layer. It was assumed that no loss of NH_4^+-N occurred either through NH_3 volatilization or NH_4^+ fixation in the clay lattice. It was also assumed that no NH_4^+-N was converted to NO_3^--N in the anaerobic soil layer.

The total loss of NH_4^+-N obtained experimentally was as high as 0.34, 1.33, 5.06, 7.15, 8.93 and 12.43 g N/m^2 occurring at 7, 15, 30, 60, 90, and 120 days incubation as compared to 44.84 g N/m^2 present at the beginning of the experiment. The loss of N as a result of diffusion from the anaerobic soil layer at the end of the 120-day incubation was 7.16 g N/m^2, compared to a total loss of 12.43 g N/m^2. This estimate is equivalent to a total NH_4^+-N loss of 124.3 kg N/ha/120 days during 4-month incubation period which amounts to about 1 kg N/ha/day. This value is higher than would occur under natural conditions, since the soil or sediment system usually does not have such a high initial concentration of NH_4^+-N. The contribution of NH_4^+-N diffusion to N loss

Figure 29. The loss of ammonium N from the flooded soil columns after different incubation periods.

at low NH_4^+-N concentration in a flooded soil or sediment system should

be approximately in the same proportion as was shown by the above cal-

culation for high NH_4^+-N concentrations.

The theoretical NH_4^+-N losses F(t) (equation [11]) in the soil

columns were calculated for three nitrification rates (k values) af-

ter 30, 60, 90, and 120 days of flooding. These data, along with

NH_4^+-N losses from the experimental soil columns, are shown in Table 2.

The F(t) values were calculated from equation [11]. The total loss of

NH_4^+-N was calculated from the product of k, t, and a. These calcula-

tions assumed that a, the depth of the aerobic soil layer, was 1.5 cm

throughout the incubation period. A D value (diffusion coefficient

of NH_4^+-N in saturated Crowley silt loam) of 0.216 cm^2/day was used in

the calculations. The k value of 3.18 $\mu g/cm^3$/day was measured in an

independent experiment; the other value of 5.00 $\mu g/cm^3$/day was chosen

on an arbitrary basis and 6.67 $\mu g/cm^3$/day was calculated from the

NH_4^+-N disappearance data in the present experiments. The theoretical

F(t) values (total amount of NH_4^+-N diffusing from the anaerobic layer

to the aerobic layer in time (t) for a k value of 5.00 $\mu g/cm^3$/day

agreed reasonably well with the corresponding values obtained from

the experimental columns for 60, 90, and 120 days. The theoretical

and experimental values for total disappearance agreed best for the

experimental k value of 6.67 $\mu g/cm^3$/day. The disappearance of NH_4^+-N

from a flooded soil system for appreciable time periods is dependent

upon the diffusive flux of NH_4^+-N from the anaerobic soil layer to the

aerobic soil layer. From equation [11], it is seen that F(t) is .

directly proportional to k. Considering that the diffusion coeffi-

cient of NH_4^+-N in water saturated soil is about 0.216 cm^2/day, the

Table 2. Comparison of NH_4^+-N loss from experimental soil columns, (see Figure 2) and theoretical loss for three k values at 30, 60, 90, and 120 days (values of a = 1.5 cm and D = 0.216 cm^2/day were assumed for theoretical calculations).

Time	k	F(t), Eq. 11	T_D, total disappearance, = kta	Nitrification of NH_4^+-N initially in aerobic layer T_D - F(t)
days	$\mu g/cm^3$/day	------------	g/m^2	----------------
		Theoretical		
30	3.18	0.76	1.43	0.67
	5.00	1.19	2.25	1.06
	6.67	1.59	3.00	1.41
60	3.18	1.81	2.86	1.05
	5.00	2.85	4.50	1.65
	6.67	3.80	6.00	2.20
90	3.18	2.94	4.29	1.35
	5.00	4.63	6.75	2.12
	6.67	6.18	9.00	2.83
120	3.18	4.12	5.72	1.60
	5.00	6.49	9.00	2.51
	6.67	8.65	12.01	3.35
Soil Columns, Figure 2:				
30		3.10	5.00	1.95
60		3.50	7.25	3.75
90		5.00	8.95	3.80
120		7.20	12.35	5.20

rate of nitrification, k, is the rate controlling factor in determin-
ing N loss.

The transport of NH_4^+-N through ionic diffusion from the anaerobic
soil layer to the aerobic soil layer can be influenced by several fac-
tors such as organic matter status of the soil, cation exchange capa-
city of the soil, presence of reduced Fe and Mn, bulk density and
moisture content. In this experiment all the above factors were con-
ducive to a high rate of NH_4^+-N diffusion. The organic matter status
of the soil was low which increased the thickness of the aerobic sur-
face soil layer. The thicker the aerobic soil layer, the greater the
nitrification of NH_4^+-N because of the greater activity of nitrifying
organisms (Ardakani et al. 1974a, 1974b). Since the anaerobic soil
layer is devoid in O_2 and NO_3^--N, Fe^{+3} and Mn^{+4} were reduced during
microbial respiration. Large quantities of reduced Fe and Mn occupy
most of the exchange complex enabling the NH_4^+-N ions to be in the soil
solution and therefore result in a high diffusion rate of NH_4^+-N in
flooded soils (Gotoh and Patrick, 1972, 1974). Low cation exchange
capacity of Crowley silt loam and water saturation of the flooded soil
columns also increased the diffusion of NH_4^+-N from the anaerobic soil
layer to the aerobic soil layer. These factors have been discussed
by several workers for ion diffusion in well drained soils (Barber,
1962; Olsen and Kemper, 1968; Gardner, 1965).

The mechanism of NH_4^+-N diffusion from the anaerobic soil layer to
the aerobic soil layer explains the large losses of applied NH_4^+-N en-
countered by Tusneem and Patrick (1971); Broadbent and Tusneem (1971);
Patrick and Gotoh (1974); Yoshida and Padre (1974). The mechanism of

NH_4^+-N loss from flooded soil or shallow lake bottoms are shown in Figure 4.

Experiment IV

Effect of Placement and Concentration of Applied Ammonium N on Nitrogen Loss From Flooded Soil.[4]

The recovery of applied N in the treatment with no added rice straw is shown in Figure 30. The labelled N fraction was determined in both organic and inorganic fractions, and the N unaccounted for was assumed to be lost from the system. A small portion of the added inorganic N was immobilized into the organic fraction and remained at a relatively constant value for all treatments during the entire incubation period. The disappearance of inorganic-N was very rapid for all the treatments. For the soil column in which NH_4^+-N was mixed throughout, the initial rate of loss was low, but rapid disappearance occurred during the latter part of incubation. The NH_4^+-N disappearance was probably due to two mechanisms: 1) NH_4^+-N already present in the aerobic soil layer was lost through nitrification and subsequent denitrification, 2) the NH_4^+-N present in the anaerobic soil layer was lost as the result of several processes occurring in sequence, namely, NH_4^+-N diffusion from the anaerobic soil layer to the aerobic surface layer, NH_4^+-N oxidation (nitrification) in the aerobic soil layer, diffusion of NO_3^--N from the aerobic soil layer down into the anaerobic soil layer, and denitrification of NO_3^--N into gaseous end products such as N_2 and N_2O in the anaerobic soil layer. No evidence of NH_3 volatilization was found.

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[4]This section is accepted for publication in Soil Science.

Figure 30. The distribution of labelled N in various N fractions of a flooded soil with no added rice straw, as influenced by the placement of ammonium N.

In treatment B where NH_4^+-N was applied to the upper 1-cm soil layer (which became the aerobic layer), initial N loss was greater than for other treatments. The NH_4^+-N probably underwent nitrification without having to diffuse from the underlying anaerobic layer to the aerobic layer and then underwent denitrification as the NO_3^--N diffused downward into the anaerobic soil layer. NH_4^+-N disappearance was rapid for treatment B during the first 90-day incubation period. When NH_4^+-N was applied to the lower 1 cm of the soil core (treatment C), the disappearance of NH_4^+-N showed a lag during the first two weeks of incubation, but later showed rapid disappearance like other treatments. The loss of N in this treatment was dependent on diffusion of NH_4^+-N to the aerobic layer because nitrification does not occur in the anaerobic soil layer (Alexander, 1965). Flooded soil incubated under an anaerobic atmosphere showed no N loss from NH_4^+-N (Patrick and Tusneem, 1972). Even though the soil column used was only 4 cm long, the aerobic soil layer was not more than 1.5 cm thick at the end of the 120-day incubation period. For treatment D where NH_4^+-N was applied to the overlying floodwater, there was a rapid loss of N due to direct nitrification of NH_4^+-N both in the aerobic floodwater and in the aerobic soil layer, followed by denitrification of NO_3^--N in the anaerobic soil layer.

The effect of added rice straw on N loss was also studied using the same four different methods of application of N to flooded soil. Addition of organic matter has been shown to immobilize part of the applied N in soils. Apparently because of this immobilization the addition of rice straw decreased the magnitude of N loss (Figure 31).

NH$_4$-N applied uniformly throughout the soil

NH$_4$-N applied to upper 1 cm soil layer

NH$_4$-N applied to lower 1 cm soil layer

NH$_4$-N applied to floodwater

Labeled Nitrogen—µg/g

Time-Days

Figure 31. The distribution of labelled N in various N fractions of a flooded soil with added rice straw, as influenced by the placement of ammonium N.

The decrease in N loss was approximately the same as the increase in N immobilization. The amount of N lost was also dependent on the placement of NH_4^+-N in the soil column. The thickness of the surface aerobic soil layer was decreased considerably by the added rice straw because of greater demand for O_2 in the surface soil layers. Nitrogen loss was lowest in treatment C where N was applied to the lower 1 cm of soil. The low N loss in this treatment was due to the fact that NH_4^+-N was an appreciable distance away from the aerobic surface layer. Nitrogen loss was high when NH_4^+-N was applied to the overlying floodwater as was also the case where the rice straw was not used. When NH_4^+-N was mixed uniformly throughout the soil (with added rice straw), a considerable loss of 52% of applied N occurred as compared to 75% of applied N loss for the same treatment with no added rice straw. Net labelled N loss as a function of time is expressed as a quadratic equation in Table 3. The N disappearance was correlated with time elapsed during incubation and the Y values show highly significant relationships in all four treatments. Similar losses of applied fertilizer N can occur under flooded field conditions. Although subsurface placement results in less N loss than surface application, band placement and deep placement of NH_4^+-N fertilizer create a concentration gradient that enhances the movement of NH_4^+-N to the surface, resulting ultimately in the loss of N.

When nonlabelled N was applied at different rates uniformly throughout the soil column (Experiment 2), similar trends in inorganic N disappearance were shown (Figure 32). The total inorganic N not recovered was directly proportional to the rate of N application (Figure 33).

Table 3. Quadratic regression equations and correlation coefficients representing the rates of NH_4^+-N loss under varying conditions in a flooded soil.

Treatment	Without Added Rice Straw	With Added Rice Straw
A. NH_4^+-N applied uniformly throughout the soil	$Y = -.004t^2 + 1.09t + 5.44$ $r = 0.982$	$Y = -.0014t^2 + 0.63t + 0.36$ $r = 0.964$
B. NH_4^+-N applied to upper 1 cm soil layer	$Y = -.005t^2 + 1.23t + 3.48$ $r = 0.999$	$Y = -.0015t^2 + 0.69t - 5.44$ $r = 0.996$
C. NH_4^+-N applied to lower 1 cm soil layer	$Y = -.004t^2 + 1.15t - 9.86$ $r = 0.997$	$Y = -.0004t^2 + 0.48t - 1.04$ $r = 0.978$
D. NH_4^+-N applied to over-lying floodwater	$Y = -.006t^2 + 1.43t - 7.49$ $r = 0.999$	$Y = -.0073t^2 + 1.51t - 10.01$ $r = 0.986$

Where Y = net loss of labelled N in g/g and t = elapsed time in days and is expressed by the equation $Y = at^2 + bt + c$. r = correlation coefficient.

Figure 32. The recovery of inorganic N, as influenced by the rate of ammonium N application and incubation period in a flooded soil.

Figure 33. The recovery of inorganic N, as influenced by the rate of NH_4^+-N application at 90- and 120-day incubation periods.

The vertical distribution of applied NH_4^+-N in the soil column at different rates of N is shown in Figure 34 for the 90-day incubation period. Very little difference with depth was observed in the distribution of NH_4^+-N where no N was applied, but increased rates of NH_4^+-N application showed a depletion of NH_4^+-N in the surface layer. The NO_3^--N concentration in the surface layer was maximum (28 μg NO_3^--N/g), where 400 μg NH_4^+-N/g of soil was applied. As pointed out above, NH_4^+-N depletion and NO_3^--N buildup in the surface layer are indicators of the processes that ultimately result in N loss by denitrification.

Increased rate of NH_4^+-N application also increased the thickness of the aerobic soil layer as shown by the redox potential profile (Figure 35). A redox potential of +300 is indicated as the transition point between aerobic and anaerobic conditions since several studies suggest that nitrate becomes unstable at about this potential (Pearsall, 1938; Aomine, 1962; Patrick, 1960). Although the reason for high concentrations of NH_4^+-N increasing the thickness of the surface aerobic layer is not known with certainty, it is very likely due to the greater production of NO_3^--N resulting from this NH_4^+-N and the effect of this NO_3^--N diffusing downward into the flooded soil on redox potential. It was shown in experiment I that NO_3^--N is present in the aerobic zone, but cannot be detected in the underlying anaerobic zone. This same effect can be observed in Figure 34 where NO_3^--N was detected at a greater depth in the high NH_4^+-N treatments. The presence of NO_3^--N in the surface soil layer oxidized Fe^{+2} to Fe^{+3} and increases the thickness of the reddish-brown aerobic layer. Very thin layer of aerobic layer was found in the treatment with no added N as compared to the treatment receiving NH_4^+-N (Figure 36).

Figure 34. The distribution of NH_4^+-N and NO_3^--N in aerobic and anaerobic soil layers at 90-day incubation period, as influenced by the rate of NH_4^+-N application to a flooded soil.

Figure 35. The effect of N application on the thickness of aerobic soil
layer after 90-day incubation period.

Figure 36. Thickness of aerobic layer at different incubation periods, as influenced by added ammonium nitrogen.

Experiment V

Effect of Long-Term Alternate Aerobic and Anaerobic Conditions on Redox Potential, Organic Matter Decomposition and Nitrogen Loss in a Flooded Soil.[5]

Redox Potential of the Soil During Long-Term Alternate Aerobic and Anaerobic Conditions:

The redox potential of the soil responded rapidly to changes in the aeration status of the soil (Figure 37A and 37F). The maximum redox potential under aerobic conditions for all treatments was approximately +600 mV. Displacement of air by Ar caused a rapid decrease in redox potential. The level to which the redox potential fell was approximately +200 mV for the treatment in which the gas was alternated every 2 days and reached lower values when the anaerobic period was extended. For the 4 and 4 day and 8 and 8 day aerobic-anaerobic periods the first decrease in redox potential was greater than subsequent decreases. In the continuously aerobic treatment the potential rose rapidly to approximately +600 mV and remained at this value throughout the 128 day incubation period. For the continuously anaerobic treatment the potential decreased rapidly to approximately -300 mV and remained constant for the entire incubation period. When the length of the aerobic-anaerobic periods was extended beyond 8 and 8 days the potential did not decrease very rapidly when Ar was bubbled through the samples. This slower initiation of reducing conditions was likely due to the presence of nitrate which had accumulated as a result of NH_4^+-N oxidation during the relatively long aerobic period. Nitrate has been shown to prevent a rapid decline in redox potential.

Figure 37A,B,C,D,E,F,G,H. Changes in redox potential of the soil as affected by alternate aerobic and anaerobic conditions.

In the soil that was maintained under a 64 and 64 day aerobic-anaerobic treatment (Figure 37F), the redox potential pattern after introduction of Ar was typical of that shown by a flooded soil containing both O_2 and nitrate (Engler, Patrick and Antie, 1974 - In preparation). Rapid depletion of the O_2 as a result of displacement by Ar and consumption by the soil caused an initial decrease in redox potential from +600 mV to +200 mV at which value the potential was stabilized for several weeks. Stabilization of the potential at approximately +200 mV was very likely due to the accumulated NO_3^--N and when this NO_3^--N was denitrified after approximately 100 days the redox potential rapidly fell to approximately -300 mV.

The redox potential values at the end of the incubation period for the various treatments are shown in Figure 38A. Since the soil was anaerobic during the last half cycle the potentials are generally reducing, except for the treatment that went through rapid aerobic-anaerobic changes and for the treatment that was maintained under continuously aerobic conditions. There appeared to be a close relationship between the redox potential at the end of incubation and the length of the aerobic-anaerobic periods with a long final anaerobic half cycle being associated with a low redox potential.

The final pH of the soil was influenced by the treatment (Figure 38B). For the soil carried through the various aerobic-anaerobic cycles the final pH was higher for the longer cycles, increasing from pH 6.5 for 2 and 2 day to 7.4 for the 64 and 64 day treatment. The controlling factor determining pH in these treatments was very likely redox potential during the final incubation period since a close inverse relationship between redox potential and pH was observed. For

Figure 38a,b. Redox potential and pH of the soil at the end of 128-day incubation period for the various treatments.

the continuous aerobic and continuous anaerobic treatments the same inverse relationship between redox potential and pH was evident. The soil continuously aerated had a lower final pH than did the original soil, probably resulting from the accumulation of NO_3^--N and SO_4^+ during the long aerobic period. Completely anaerobic conditions resulted in a pH of 7.8, a value somewhat higher than is usually encountered when acid soils are flooded. It should be noted that continuous passage of Ar through the suspension prevented precipitation of carbonate compounds which function to stabilize pH near the neutral point. This suggests that the pH value of flooded soils is sensitive to loss of CO_2. The pH of most reduced soils equilibrated with CO_2 at 1 atm. is 6.1 (Ponnamperuma, Castro and Valencia, 1969).

Decomposition of Organic Matter as a Result of Alternate Aerobic and Anaerobic Conditions:

The aeration status of the soil had a marked effect on organic matter breakdown. Carbon dioxide evolution for the various treatments is shown in Figure 39 (A-H). There was little difference in CO_2 evolution patterns for aerobic-anaerobic cycles ranging from 2 and 2 to 32 and 32 days. Carbon dioxide evolution was decreased considerably, however, when the cycle was increased to 64 and 64 days. Aerobic conditions for the entire incubation resulted in approximately the same loss of CO_2 as was observed for most of the aerobic-anaerobic treatments. For the soil kept under continuous anaerobic conditions CO_2 evolution was decreased to approximately half the values obtained for the other treatments. Organic matter breakdown is known to be slower under anaerobic conditions than under aerobic conditions. (Acharya,

Figure 39a,b,c,d,e,f,g,h.   Rate of decomposition of organic matter as influenced by alternate aerobic and anaerobic conditions.

1935; Alexander, 1961; Tenny and Waksman, 1930). Since C can also be lost from anaerobic soil as CH_4, which was not measured in this experiment, total C loss was calculated from total C content at the beginning and end of the experiment. Carbon loss as determined from dry combustion analysis of organic C at the beginning and end of the experiment showed remarkable agreement with the values obtained by CO_2 evolution (Figure 40A), indicating little C loss as CH_4. The only treatments that showed much discrepancy in C loss between the two methods was the 8 and 8 day aerobic-anaerobic periods and the continuously anaerobic treatment. The slightly greater loss of C for the continuously anaerobic treatment may have been due to CH_4 formation.

Loss of Added and Native N as a Result of Alternate Aerobic and Anaerobic Conditions:

The loss of N as a result of the various aerobic-anaerobic cycles is shown in Table 4 and Figure 40B. The greatest loss of N took place in the treatments that alternated between aerobic and anaerobic conditions every 2 days. Approximately one-fourth of the total N (24.3%) was lost during 32 of these cycles. Increasing the durations of the aerobic-anaerobic periods decreased the loss of N with approximately 13% loss for the 64 and 64 day treatment. Almost no N was lost in the continuously anaerobic treatment while 7%

(64 µg/g) was lost in the continuously aerobic treatment. N is sometimes lost from apparently well aerated soils because of the presence of anaerobic microzones where denitrification can take place, but the continuous stirring and bubbling of air through the soil suspension should have prevented such anaerobic microzones from being established. This small loss of N may have resulted from chemical decomposition of

Table 4. Changes in total nitrogen as result of alternate aerobic and anaerobic conditions

Length of aerobic period (CO_2-free air) (days)	Length of anaerobic period (Ar) (days)	Number of complete cycles during incubation period	Total Nitrogen* ($\mu g/g$)		
			At the end of experiment	Net Loss	Loss (%)
2	2	32	704	226	24.3
4	4	16	717	213	22.9
8	8	8	730	200	21.5
16	16	4	744	186	20.0
32	32	2	762	168	18.0
64	64	1	810	120	12.9
128	-	Completely aerobic	866	64	6.9
-	128	Completely anaerobic	925	5	0.5

* At the beginning of the experiment soils in all treatments contained 930 μg N/g of soil.

Figure 40a,b. Total carbon and total nitrogen content of the soil at the
end of 128-day incubation period as influenced by various treatments.

of NO_2^--N. Some workers have shown that N losses during the nitrification process are the consequence of chemical decomposition of NO_2^--N under acid soil conditions (Tyler and Broadbent, 1960; Smith and Clark, 1960; Cady and Bartholemew, 1963). No decomposition of NO_2^--N was observed by Greenwood (1962) and Meek and Mackenzie (1965) in alkaline soils. Nitrite formed during the nitrification process might suffer some loss under the low pH conditions of the continuously aerated treatment.

In all of the aerobic-anaerobic cycles the redox potential fluctuated between values that were oxidizing enough to support nitrification and reducing enough (below approximately +340 mV) to support denitrification (Pearsall, 1938; Patrick, 1960). The 2 and 2 day aerobic-anaerobic cycle was chosen because it was thought that the duration of the anaerobic half cycle would be too short for the redox potential to fall below the critical value necessary for NO_3^--N reduction. The significant loss of N from the soil subjected to the 2 and 2 day aerobic-anaerobic periods indicates, however, that a 2 day period was long enough for nitrification to occur when air was bubbled through the suspension. A 2 day period was also long enough to support denitrification when Ar was bubbled through. Loss of N during short aerobic and anaerobic periods may be of more significance in nonflooded soils where temporary anaerobic conditions can occur than in flooded soils.

The amount of added labelled N remaining in the soil following incubation for 128 days for the various aerobic-anaerobic treatments is shown in Figure 41A. Less than half of the added labelled N (37%

Figure 41a,b. Amount of labelled nitrogen and inorganic nitrogen remaining in the soil following incubation for 128 days under alternate aerobic and anaerobic conditions.

in the case of the 2 and 2 day cycle) was recovered in all of the treatments where the soil underwent alternate aerobic and anaerobic incubation. Almost all of the N that was recovered was present in the organic fraction. This was especially true for the treatments with short aerobic-anaerobic periods; no inorganic N was detected in the 2 and 2, 4 and 4 and 8 and 8 day treatments. It is likely that N loss would have been greater if an appreciable part of the added labelled N had not been incorporated into microbial tissue, from which release by decomposition was slow.

All of the added labelled N was recovered in the completely anaerobic treatment, either as organic N or as NH_4^+-N. The completely aerobic treatment showed a loss of 18 percent of the added 100 µg/g labelled NH_4^+-N. As pointed out above, this loss could have resulted from denitrification in a system being continuously stirred in the presence of O_2, or as is also likely, from chemical breakdown of NO_2^--N during the nitrification process. All of the inorganic N recovered in the continuously aerobic treatment was in the nitrate form.

Inorganic N (labelled and unlabelled) at the end of the experiment for the various treatments is shown in Figure 41B. Little inorganic N, either labelled or unlabelled, was recovered at the end of incubation for treatments with aerobic-anaerobic cycles shorter than 32 and 32 days. For all of the alternating aerobic-anaerobic treatments except the 2 and 2 day treatment, all of the inorganic N was present as NH_4^+. A trace of NO_3^--N was present for the 2 and 2 day treatment. It should be remembered that the last half cycle was under anaerobic conditions. Completely aerobic or completely anaerobic con-

ditions resulted in accumulation of appreciable inorganic N. This N

was present as NO_3^- in the aerobic treatment and as NH_4^+ in the anaero-

bic treatment. For both these treatments and for the alternate aero-

bic and anaerobic treatments which had inorganic N present at the end

of incubation, approximately one-third of the inorganic N was derived

from the labelled NH_4^+-N and two-thirds from reactive soil N.

This study shows that increasing the frequency of changing from

aerobic to anaerobic soil conditions to 2 days aerobic and 2 days

anaerobic increases the loss of N. This loss was especially severe

for added NH_4^+-N with 63 percent of added labelled NH_4^+-N being lost

for the 2 and 2 day treatment during a 128 day incubation. More N

would possibly have been lost except for immobilization of part of the

added NH_4^+-N by added rice straw.

Experiment VI

Effect of Short-Term Alternate Aerobic and Anaerobic Conditions on
Redox Potential and Nitrogen Loss in a Flooded Soil.[6]

Redox Potential of the Soil During Short-Term Alternate Aerobic and
Anaerobic Conditions in Flooded Soil:

The effect of frequent changes in aerobic-anaerobic conditions

on redox potential are shown for the first 10 days of each treatment

in Figure 42. The redox potential decreased to approximately +300 mV

during the anaerobic period and increased to approximately +600 mV

during aerobic period for the 6 and 6 hour treatment. The minimum re-

dox potential recorded was less than +300 mV during the anaerobic per-

iod for the 12 and 12, 24 and 24, and 48 and 48 hour treatments. Re-

Figure 42. Changes in redox potential for 10-day period, as influenced
by frequent changes in aerobic and anaerobic conditions in Experiment
VIa.

Figure 43a. Maxima and minima of redox potential values reached at the end of each aerobic and anaerobic period during 128-day incubation in Experiment VIa.

Figure 43b. Maxima and minima of redox potential values reached at the end of each aerobic and anaerobic period during 128-day incubation in Experiment VIa.

dox potential values below approximately +340 mV have been reported to be favorable for denitrification (Pearsall and Mortimer, 1939; Patrick, 1960). The maxima and minima of redox potentials at the end of each aerobic period and at the end of each anaerobic period are presented in Figures 43a and 43b for the 128-day incubation period. The redox potential recorded at the end of each aerobic period was about +640 mV for all treatments, whereas the redox potential at the end of each anaerobic period was as low as +300 mV during the early part of incubation, but increased to about +500 mV during the latter part of incubation in the 6 and 6 hour treatment, probably as a result of energy depletion. Similar trends were observed for other treatments having frequent changes in aerobic and anaerobic conditions.

The redox potential measured during alternate aerobic and anaerobic conditions for 64 days in Experiment VIa are presented in Figure 44. In the treatment where the change from aerobic to anaerobic conditions was made every 1.5 hours, the redox potential reached maxima of approximately +640 mV during the aerobic periods. The minimum redox potential values reached at the end of the 1.5 hour anaerobic period was approximately +400 mV during the early part of incubation and approximately +550 mV during the latter part of incubation. Similar trends in the redox potential were also obtained for the 3 and 3 hour treatment.

At the beginning of each aerobic period the redox potential increased rapidly when air (21% O_2) was introduced and the potential remained relatively stable during the aerobic period. When Ar was bubbled into the soil suspension at the beginning of each anaerobic period, however, the redox potential decreased very slowly and was some-

Figure 44. Maxima and minima of redox potential values reached at the end of each aerobic and anaerobic period during 64-day incubation in Experiment VIb.

what erratic. These results indicate rapid oxidation of reduced components upon introduction of O_2 into the anaerobic soil and slow reduction of oxidized components upon introduction of Ar in the aerobic soil. It is possible that the oxidation processes were largely chemical, such as oxidation of Fe^{2+} to Fe^{3+} by O_2, while the reduction processes were biological and therefore somewhat slower. Also, nitrate produced in the soil during the aerobic period tends to keep the redox potential at a high value and results in a slow decrease in redox potential.

Loss of Native and Added N as a Result of Frequent Changes in Aerobic and Anaerobic Conditions:

Loss of total N (native + added) as influenced by frequent changes in aerobic and anaerobic conditions for two different incubation periods are presented in Table 5. The N losses were as high as 37.2% of the total N in short-term cycles of 6 hour alternate aerobic and anaerobic conditions followed by 32.1, 24.9, and 25.7% for 12 hour, 24 hour, and 48 hour alternate aerobic-anaerobic cycles respectively, for a period of 128 days (Experiment VIa). In all the treatments no inorganic N (NH_4^+ or NO_3^-) was recovered at the end of the incubation period, showing that part of the added N plus mineralized NH_4^+-N was lost as a result of nitrification (during aerobic period) and denitrification (during anaerobic period) and the remaining N was incorporated into microbial tissue. The redox potential fell below approximately +340 mV during the anaerobic period during the early part of the incubation period, indicating favorable conditions for denitrification.

Large losses of native and added N were also encountered in Experiment VIb, where short-term cycles were as frequent as 1.5 hour

Table 5. Changes in total nitrogen as influenced by frequent changes in aerobic and anaerobic conditions in flooded soil.

Length of aerobic period (21% O_2) (hours)	Length of anaerobic period (Ar) (hours)	Number of complete cycles during incubation period	Total Nitrogen (μg/g)			
			At beginning of experiment	At end of experiment	Net Loss	Loss (%)
EXPERIMENT VIa						
6	6	256	936.0 ± 32.1	588.2 ± 9.8	347.8	37.2
12	12	128	936.0 ± 32.1	636.5 ± 38.9	300.0	32.1
24	24	64	936.0 ± 32.1	702.7 ± 38.8	233.5	24.9
48	48	32	936.0 ± 32.1	695.8 ± 9.8	240.2	25.7
EXPERIMENT VIb						
1.5	1.5	512	962.0 ± 30.1	780.6 ± 29.3	181.4	18.9
3	3	256	962.0 ± 30.1	808.8 ± 9.7	153.2	15.9
6	6	128	962.0 ± 30.1	793.8 ± 19.4	168.2	17.5
*6	*6	128	962.0 ± 30.1	726.4 ± 29.3	235.6	24.5

*Nitrate N (100 μg/g) was added instead of ammonium N at the beginning of the experiment.

aerobic and 1.5 hour anaerobic. The losses were 18.9, 15.9, and 17.5% of total N for 1.5 hour, 3 hour and 6 hour alternate aerobic and anaerobic conditions respectively, during a period of 64 days. For the 6 hour treatment where nitrate N instead of NH_4^+-N was used, the loss was 24.5% of total N. Changes in inorganic N (unlabelled + labelled N) at several incubation periods are presented in Figure 45 for these treatments. Ammonium N disappeared rapidly from the soil suspension. There was a slight buildup of NO_3^--N, especially in the 1.5 hour and 3 hour treatments, but not enough to compensate for the NH_4^+-N decrease. Nitrate was apparently being formed and lost from the system. For the treatment with 6 hours aerobic-anaerobic periods, the nitrate was probably lost by denitrification. Denitrification may have been responsible for N loss in the soils subjected to shorter aerobic-anaerobic periods, although the fact that the redox potential did not decrease below about +500 mV suggests that some other mechanism may have been involved. Nitrite formed during the nitrification process may have undergone chemical decomposition. The maximum NO_2^--N concentration found in the treatments, however, was 0.5 $\mu g/g$ of soil.

The amount of added labelled N remaining in the soil at the end of the 128-day incubation period (Experiment VIa) is shown in Table 6. For all treatments no labelled N was recovered in the inorganic fraction at the end of the incubation period. The net loss of added labelled N (not recovered in either organic or inorganic fractions) was high, 71.2, 66.3, 65.0, and 61.3% of added labelled N for 6 hour, 12 hour, 24 hour, and 48 hour alternate aerobic and anaerobic conditions, respectively. The added labelled N was subjected to nitrification during the aerobic period and denitrification during the anaerobic

Table 6. Fate of applied $^{15}NH_4$-N (100 µg N/g soil) in various treatments following frequent changes in aerobic and anaerobic conditions in a flooded soil during 128-day incubation period.

N-Fraction	Treatments (hours)			
	6-6	12-12	24-24	48-48
	Labelled N µg/g			
Organic N	28.8 ± 0.85	33.7 ± 1.05	35.0 ± 0.50	38.7 ± 0.45
Inorganic N*	---	---	---	---
N-Unaccounted for	71.2	66.3	65.0	61.3

*Samples did not have enough total inorganic N (<1.2 µg/g) for labelled N analysis in the mass spectrometer.

Figure 45.   Changes in inorganic nitrogen as influenced by frequent
changes in aerobic and anaerobic conditions during 64-day incubation
in Experiment VIb.

period.

The amount of labelled N recovered at the end of the 64-day incubation period for Experiment VIb is shown in Table 7. The net loss of labelled N was 41.3, 28.9, and 54.6% of applied N for the 1.5 hour, 3 hour, and 6 hour treatments, respectively. Under complete aerobic conditions for 128 days, 18% of applied NH_4^+-N was not recovered, indicating that mechanisms other than denitrification were probably involved in N loss. No NH_3 was recovered in the traps provided, showing that NH_3 volatilization was not responsible.

The results of this study show that increasing the frequency of changing from aerobic to anaerobic conditions from 48 hours aerobic and 48 hours anaerobic to 6 hours aerobic and 6 hours anaerobic increased the loss of total N and labelled inorganic N. In an additional experiment where even more frequent changes in aerobic-anaerobic conditions were made, losses were less for a 3 hour aerobic-3 hour anaerobic treatment than for the 6 hour treatment. Increasing the frequency of change of aerobic-anaerobic conditions to 1.5 hours, however, resulted in greater N loss than for the 3 hour treatment. Nitrogen loss was apparently due to nitrification during the aerobic period followed by denitrification during the anaerobic period. Nitrite formation and chemical decomposition of NO_2^--N may also have been involved in N loss, especially for the more frequent changes in aerobic-anaerobic conditions.

Table 7. Fate of applied $^{15}NH_4$-N (100 µg N/g soil) in various treatments following frequent changes in aerobic and anaerobic conditions in a flooded soil, during 64-day incubation period.

	Treatments (hours)							
	1.5-1.5		3-3		6-6		**6-6	
N-Fraction	Labelled N µg/g							
Organic-N	41.90	2.31	46.40	0.77	45.4	2.51	6.6	3.0
Ammonium-N	12.72	0.76	16.61	0.76	*---		---	
Nitrate + Nitrite-N	6.60	1.74	12.53	1.40	*---		---	
N-Unaccounted for	41.3		28.9		54.6		93.4	

* Samples did not have enough ammonium or nitrate N (<0.36 µg/g) for labelled N analysis in the mass spectrometer.

** Nitrate N (100 µg/g) was added instead of ammonium N at the beginning of the experiment.

SUMMARY AND CONCLUSIONS

Tracer studies on nitrification-denitrification reactions were
conducted under laboratory conditions on Crowley silt loam. Ammon-
ium sulfate enriched with ^{15}N was used as a tracer to follow the path
of applied nitrogen.

The roles of NH_4^+-N diffusion, nitrification, NO_3^--N diffusion, and
denitrification in controlling N loss from continuously flooded soil
and water bodies were evaluated. All four processes were shown to be
occurring sequentially in a flooded soil incubated for 60 days using
labelled N. The slow rate of NH_4^+ diffusion from the anaerobic soil
layer to the aerobic soil layer, and the slow rate of nitrification in
the aerobic soil layer indicate that these two processes are limiting
steps in controlling N loss. Nitrate diffusion from the aerobic soil
layer to the underlying anaerobic soil layer and denitrification in
the anaerobic layer were found to proceed at faster rates and are not
likely to limit N loss in a flooded soil. These processes accounted
for a loss of 35.8% of the 200 μg labelled NH_4^+-N per g of soil ini-
tially applied during an incubation period of 60 days.

Ammonium nitrogen in a flooded soil or sediment exposed to O_2
from the water column undergoes sequential nitrification and denitri-
fication. Increasing the concentration of O_2 in the atmosphere above
floodwater increased applied NH_4^+-N loss. Oxygen aided in development
of a thick aerobic layer, thus increasing the zone of nitrification.

134

The final gaseous end products of nitrification-denitrification were found to be mainly N_2, however small amounts of N_2O were also detected. Ammonium in the aerobic surface layer of soil or sediment is nitrified, and the resulting concentration gradient between this layer and the underlying anaerobic layer causes NH_4^+ to diffuse upward into the aerobic layer where it also undergoes nitrification. Nitrate produced in the aerobic layer then diffuses down into the anaerobic layer where it is denitrified to N_2 and N_2O. If O_2 is absent or limiting, nitrification will either not occur or will occur at a lower rate, resulting in a small amount of NO_3^--N available for the denitrification process.

The role of NH_4^+-N diffusion in a flooded soil on N loss through the nitrification-denitrification process was investigated under laboratory conditions. The distribution of applied NH_4^+-N in both the aerobic and anaerobic soil layers of a flooded soil was experimentally determined and compared with the values obtained from theoretical equations. The total loss of NH_4^+-N from the flooded soil system (15-cm depth) by nitrification-denitrification was equivalent to 12.43 g N/m^2 for a 120-day incubation period when the initial concentration of NH_4^+-N was 44.84 g N/m^2. Diffusion of NH_4^+-N from the anaerobic soil layer to the aerobic soil layer accounted for more than 50% (7.16 g N/m^2) of the total NH_4^+-N loss with the remainder being lost from NH_4^+-N originally present in the aerobic layer. The NH_4^+-N that diffused upward into the aerobic soil layer was nitrified to NO_3^--N, which readily diffused back down into the anaerobic soil layer and was subsequently denitrified. The experimental distributions of NH_4^+-N were not in close agreement with calculated distributions in the surface aerobic soil layer but were in close agreement in the anaerobic soil layer. It is

possible that the rate constant (k) for NH_4^+-N oxidation varied considerably with depth in the aerobic soil layer and thus resulted in the disagreement. The total NH_4^+-N loss calculated from the experimental distribution tended to agree with the values obtained theoretically from rate constant (k) values of 3.18, 5.00, and 6.67 $\mu g/cm^3$/day. The first rate constant value was obtained from an independent experiment (for same soil) and the second from matching the concentration of NH_4^+-N at the aerobic-anaerobic layer interface of the theoretical and experimental distribution at 90 days after flooding and the third from the NH_4^+-N disappearance in the aerobic soil layer of the soil columns described in this study. These rate constants indicate that the rate of nitrification is one of the factors controlling N loss from flooded soil.

The effect of placement and concentration of NH_4^+-N in a flooded soil on N loss was investigated. Applied labelled NH_4^+-N was rapidly lost from the soil system during a 4-month incubation period regardless of where NH_4^+-N was applied in the soil column. Nitrogen loss was lower when N was placed in the anaerobic zone than when placed at the aerobic surface. Addition of rice straw decreased N loss where N was applied to the soil, but did not show any difference where N was applied to the overlying floodwater. Increasing the concentration of NH_4^+-N in the soil increased the N loss and also the thickness of the aerobic soil layer. Measurement of N distribution in the soil column showed very little accumulation of NO_3^--N in the aerobic soil layer; increasing the amount of NH_4^+-N applied in the anaerobic soil layer; however, increased the amount of NO_3^--N in the aerobic layer.

The effect of several cycles of varying length of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and loss of added and native N was investigated in a flooded soil incubated for 128 days. Redox potential decreased rapidly when air was replaced with argon for the short-time cycles, but decreased more slowly where the aerobic period was long enough to permit buildup of nitrate. The minimum redox potential reached during the anaerobic period was generally lower for the longer cycles, but in all cases was low enough for denitrification to occur. Rate of decomposition of organic matter was faster in the treatments with a greater number of alternate aerobic and anaerobic periods. Total N (native and applied) losses as high as 24.3% occurred in the treatment with the maximum number of cycles and with alternate aerobic and anaerobic periods of 2 and 2 days. Increasing the durations of the aerobic-anaerobic periods decreased the loss of N. A maximum loss of 63.0 percent of applied labelled NH_4^+-N resulted from the shortest (2 and 2 day) aerobic and anaerobic incubation. For soil undergoing frequent changes in aeration status the only labelled N that remained at the end of incubation was found in the organic fraction. Loss of N may have been even greater if labelled inorganic N had not been immobilized by microorganisms decomposing the added rice straw. The greater loss of N resulting from the 2 and 2 day aerobic-anaerobic incubation shows that, in soils where the redox potential falls low enough for denitrification to occur, increasing the frequency of changing from aerobic to anaerobic conditions will increase the loss of N.

The effect of frequent changes in aerobic and anaerobic conditions

on redox potential and nitrogen (N) loss in a flooded soil was investigated under laboratory conditions. Increasing the frequency of changing from aerobic to anaerobic conditions from 48 hour aerobic-48 hour anaerobic to 6 hour aerobic-6 hour anaerobic increased N loss. A separate experiment showed that losses were somewhat less when the frequency was increased from 6 hour aerobic-6 hour anaerobic to 3 hour aerobic-3 hour anaerobic, but the loss of N again increased when the frequency was further increased to 1.5 hour aerobic-1.5 hour anaerobic. Nitrogen losses were due to alternate nitrification (during aerobic period) and denitrification (during anaerobic period) possibly coupled with chemical decomposition of NO_2^--N at the greater aerobic-anaerobic frequencies.

The results obtained in the present experiments allow to make two general conclusions. These studies show that nitrogen losses in flooded soil can occur under two conditions:

(1) The NH_4^+-N in a flooded soil readily undergoes nitrification (3.18 $\mu g/cm^2$/day) in the aerobic layer, the NO_3^--N formed in the aerobic layer readily diffuses (1.33 cm^2/day) into the anaerobic layer, where it undergoes denitrification (15 $\mu g/cm^3$/day) into gaseous end products, such as N_2 and N_2O. This results in the concentration gradient of NH_4^+-N between the aerobic layer and the underlying anaerobic layer causes NH_4^+-N to diffuse upwards (0.216 cm^2/day) and is subjected to nitrification-denitrification reactions. All these processes are shown in Figure 46.

(2) The NH_4^+-N in a flooded soil readily undergoes nitrification when the soils are drained or an aerobic atmosphere is created. Upon flooding or when an anaerobic atmosphere is created, the NO_3^--N

Figure 46. Kinetics of the processes controlling nitrogen loss in flooded soil.

is lost through denitrification. This process of alternate aerobic and anaerobic conditions stimulated the applied and native nitrogen loss.

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VITA

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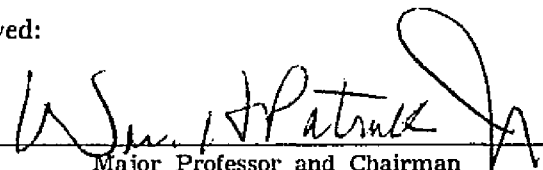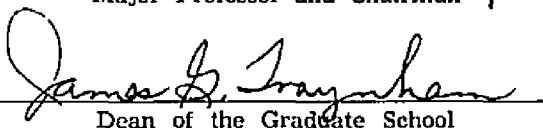
EXAMINATION AND THESIS REPORT

Candidate: Konda Rameshwer Reddy

Major Field: Agronomy

Title of Thesis: Nitrification-Denitrification Reactions in Flooded Soil
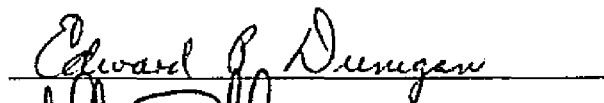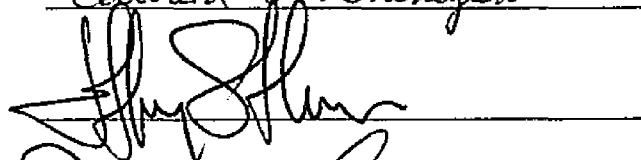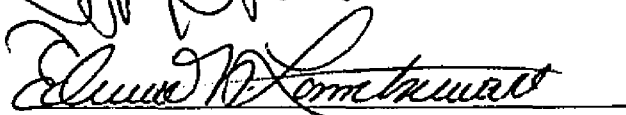
Approved:

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Major Professor and Chairman

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Dean of the Graduate School

EXAMINING COMMITTEE:

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Date of Examination:

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July 6, 1976