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Concentration and Movement of Oxygen as Related to Absorption of Ammonium and Nitrate-Nitrogen by Rice.

William Hardy Patrick
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**CONCENTRATION AND MOVEMENT OF OXYGEN AS RELATED
TO ABSORPTION OF AMMONIUM AND NITRATE NITROGEN BY RICE**

A Dissertation

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy**

in

The Department of Agronomy

by
William Hardy Patrick, Jr.
B. S., Louisiana State University, 1950
M. S., Louisiana State University, 1951
June, 1954

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ABSTRACT

A study has been made of the effects of different oxidation levels of the root substrate on the growth of rice, on the absorption of ammonium and nitrate nitrogen by rice, and on the stability of nitrate and ammonium nitrogen.

Two greenhouse experiments using Crowley silt loam, a field experiment at the Rice Experiment Station, and several laboratory experiments were conducted during this study. The growth, nitrogen contents, and potassium contents of the rice plants were measured. The nitrate nitrogen, ammonium nitrogen, and dissolved oxygen in the soil and flood water, the Eh of the soil, and the loss of ammonia by volatilization from the soil were determined. In addition to these measurements, a method was developed for determining the rate of consumption of oxygen by a soil.

Bubbling oxygen up through the flood water in rice cultures produced a significant increase in the growth of rice in a greenhouse experiment on Crowley silt loam. No increase in the free oxygen content of the soil was measured as a result of the addition of oxygen, however. The beneficial effects of the oxygen to the rice were restricted to the roots concentrated at the soil surface. Measurements made on a Crowley silt loam showed that the

rate of reduction of oxygen by the soil was increased by the submergence of the soil and by the addition of organic matter to the submerged soil. There was probably competition between the rice roots and the reduced substances in the soil for the oxygen supplied by diffusion through the flood water from the atmosphere. Much of the oxygen that diffused to the soil-water interface was not available to the rice plant. This lack of availability may have been due to the slow rate of diffusion of oxygen through the soil, the reducing conditions in the soil, or both.

Flooding the soil caused a decrease in the oxidation-reduction potential, or E_H , of the soil. The rate of decrease of the E_H was highest where organic matter was added to the soil. The oxygen concentration of the soil suspension was closely related to the oxidation-reduction potential of the suspension. A linear relationship was obtained when the E_H was plotted against the logarithm of the oxygen concentration.

Ammonium nitrogen was found to be more readily absorbed by rice than nitrate nitrogen both in the soil cultures and the nutrient solutions regardless of the oxidation level of the root medium. There did not appear to be any specific interaction between any of the oxidation treatments and the absorption of either nitrate or ammonium nitrogen by rice in any soil experiment. An increase in the rate of absorp-

tion of ammonium nitrogen due to the addition of oxygen occurred in the nutrient solution experiment.

The application of organic matter to a Crowley silt loam enhanced the loss of ammonia by volatilization. This loss of ammonia was associated with a lowered E_H , an increased pH, and flood water temperatures as high as 100° F.

INTRODUCTION

Rice is the principal food of a majority of the world's population. The nutrition of rice is very important since so many people depend on this cereal for not only their income but for their very existence. In most of the areas of the world where rice is produced extensively, nitrogen is the nutrient most commonly limiting its maximum growth. Because of this fact, a great amount of experimental work has been done on nitrogen utilization by rice. Much of this work has consisted of measuring the crop's response to different forms and amounts of nitrogen. In spite of the attention which the nitrogen fertilization of rice has received, the response made by rice to nitrogen fertilizer is comparatively less than the response of other cereals to nitrogen fertilizer.

Rice is generally grown on soils which remain submerged during most of the growing season. The submergence of the soil causes a lowering of the oxidation-reduction potential, or E_H , of the soil, an increase in alkalinity due to the hydrolysis of minerals, a low free oxygen content, and other conditions associated with poor aeration. The poor response of rice to nitrogen may be associated with some of the conditions brought about by the submergence of the soil. The reducing conditions in the soil affect the stability of nitrate and ammonium nitrogen.

Under reduced conditions nitrate nitrogen is reduced to nitrites, other lower oxides of nitrogen, free nitrogen, or ammonia. Some of these forms of nitrogen are of doubtful value as a nutrient for rice and some are subject to loss from the soil and water system. Ammonia may be lost from a submerged soil, particularly if it tends to accumulate at reactions above pH 7.0.

The purpose of this study has been, first, to study the effects of different oxidation levels of the root substrate on the growth of rice, second, to study the absorption of nitrate and ammonium nitrogen by rice as influenced by different oxidation levels of the root substrate, and third, to study the stability of nitrate and ammonium nitrogen under submerged soil conditions.

REVIEW OF LITERATURE

Early experiments have demonstrated that oxygen is necessary for the absorption of nutrient ions by plants. Hoagland and Broyer (19) and other workers have found that salt accumulation by excised barley roots ceased in non-aerated cultures. The root cells exhibited a very low permeability to nutrient salts in anaerobic cultures since very little absorption of salt by the plant occurred even though there was a concentration gradient favoring the entry of the ions into the plant.

Although the dependence of nutrient ion absorption by plants on the presence of oxygen in the root substrate has been established, the exact mechanism of this process is not definitely known. The theory of ion uptake as visualized by Lundegardh (29) is essentially anionic in nature. Electrons are transferred from inside the respiring cell to the outside medium by means of a cytochrome-cytochrome oxidase system. The movement of the electrons outward is accompanied by a simultaneous transfer of anions into the cell. Anions are thus transported from a high oxidation level outside the plant to a lower oxidation level inside the cell. The anions are accumulated inside the plant by being successively exchanged for electrons along a cytochrome bridge through the cytoplasm. Ferric and ferrous iron are visualized as being oxidized and reduced components of this cytochrome system.

The electrons which are moved across the cell boundary out of the cell into the root environment are taken from the hydrogen atoms of the carbohydrate energy source. Hydrogen ions thus accumulate within the cell. These hydrogen ions are exchanged for metallic ions outside the cell, which accounts for the entry of cations into the plant. Cation uptake is therefore an exchange reaction between hydrogen ions within the cell and cations outside the cell, whereas anion uptake is directly dependent on cell respiration. This has been shown by Lundegardh and Burestrom (31), who found that the total respiration increases or decreases with anion absorption but was mostly independent of cation absorption.

According to the Lundegardh concept (29), hydrogen ions and electrons are part of the intermediate products of the cell's metabolism. These hydrogen ions and electrons unite with free oxygen to form water which is one of the metabolic end products.

Since cation uptake is an exchange mechanism, cations should be able to move out of the root cell back into the soil as well as from the soil into the root cell. Jenny, et al. (21), using radioactive isotope tracers, have demonstrated that cations can move from the root cells of a plant back into the surrounding substrate after they have been absorbed.

If all of the respiratory oxygen consumed were used in ion absorption, four equivalents of anions could be absorbed

during the reduction of one mole of oxygen. Four electrons from inside the cell would be required to reduce an oxygen molecule and the equivalent in anions would therefore move into the cell as the electrons were being moved outward. This ratio of anions absorbed to oxygen consumed was investigated by Robertson and Wilkins (40). The highest ratios found by these workers were in storage tissues and segments of roots. The ratios ranged between three and four. Although Burstrom (9) contends that there can be no stoichiometric relationship between ion accumulation and oxygen consumed, Lundegardh (30) found for healthy wheat roots in 0.05 molar potassium chloride that the total anion respiration uses one-fourth of the oxygen consumed for active anion absorption, one-fourth for the internal transport of inorganic anions and one-half for the movement of "native" anions. These "native" anions are most likely the anions of organic acids which participate in aerobic respiration. According to Lundegardh therefore, the ratio of equivalents of oxygen consumed to anions absorbed is 1 in healthy wheat seedling roots since three-fourths of the anion respiration is concerned with the movement of the organic and inorganic anions within the plant.

The effect of free oxygen on the growth and metabolism of plants has been studied by many workers. Cannon (19) investigated the response of several different species of plants to different oxygen levels of the root zone. He found with

most of the plants studied that oxygen did not limit growth when present in the root zone in concentrations around 10 per cent of the total soil air. The importance of temperature on the demand for oxygen was also noted by Cannon. He found that the oxygen requirement of the plants he studied increased with temperature. This was explained as being due to the fact that the solubility of oxygen in the soil solution is inversely related to the temperature. Russell (42) observed that, although the decreased solubility of oxygen at higher temperatures may be a factor in this increased demand for oxygen, an important part in this increased demand is due to an increase in the rate of respiration. Cannon (10) also found that rice seedlings grew in cultures in which the oxygen concentration of the aerating gas was 0.5 per cent, although the growth was slow.

Erickson (13) grew tomatoes in aerated and nonaerated cultures for five weeks. In the aerated cultures the oxygen concentration was 8 parts per million. In the nonaerated cultures exposed to the air, the oxygen concentration decreased slowly in five weeks to less than 1 part per million. Increasing the oxygen concentration of the nutrient solutions from 0.4 parts per million to 8 parts per million resulted in increased growth and root development of the tomato plants. Similar responses to oxygen have been obtained with soybeans (15, 45), barley (3), wheat (49), rice (49, 28), and oats (15).

The role of free oxygen in the nutrition of rice has been investigated by several workers. Working with rice and barley,

Vlaminis and Davis (51) found that the shoot growth of rice was highest at low oxygen tensions of the aerating gas. The root and shoot growth of barley and the root growth of rice diminished considerably when the oxygen concentration was decreased. The ratio of shoot to root growth of the rice was therefore increased as the oxygen content of the nutrient solution fell.

The possibility that rice may be able to carry on some of its respirational functions without oxygen has been investigated by Taylor (49). He compared the growth, aerobic respiration, and anaerobic respiration or fermentation of wheat and rice. As the oxygen concentration of the nutrient substrate was decreased, the growth of wheat was more adversely affected than that of rice. At the low oxygen levels at which rice made the better growth, the rice plants released more energy by fermentation than the wheat plants. This indicated to Taylor that at least part of the energy needed by rice to carry on its growth processes may be obtained through anaerobic respiration. Wheat was found to have a stronger total respiratory system than rice, but the rice plants possessed a much stronger fermentation, or anaerobic, respiration system. In fact, the fermentation system of rice was more efficient than its aerobic respiration system. When the oxygen tension of the root substrate was decreased, there followed a rapid decrease in the rate of total energy released. The total energy released under completely anaerobic conditions for rice was one-sixth and for

wheat one-fifteenth that obtained when air was used to aerate the cultures. The smaller decrease in the energy released by rice was credited to its fermentation system. Taylor, however, did not take into account the possibility that oxygen may have diffused through the rice stems more readily than through the wheat stems.

The ability of rice to obtain the oxygen necessary for root respiration from the atmosphere by transmittal down through the plant has been demonstrated by Raalte (37). By measuring the oxygen content of the air spaces of the roots, Raalte found that there was a concentration gradient of oxygen from the base of the root to the tip. This gradient was from 9-14 per cent oxygen in the basal portion to 8.1-2.1 per cent oxygen in the root tip. The oxygen concentration of the roots was unaffected by growing the plants in a reducing solution or by bubbling nitrogen gas through the medium. This indicates that the oxygen did not come from the root medium. Raalte also noted that photosynthesis did not increase the oxygen concentration of the roots.

A study of the swamp plant, saw grass, Cladium mariscus, by Conway (11) showed that specialized tissues exist in some plants for the transmittal of oxygen. This plant, whose habitat is the swamps and bogs of England and the fresh to brackish marshes of the Gulf Coast region of North America, grows with its roots completely submerged. Conway has shown that a specialized structure, or aerenchyma, is present in

this plant and is capable of transmitting oxygen from the atmosphere to the roots of the plant. The oxygen content in the roots of this plant was maintained at as high a level as 15 per cent by the diffusion of oxygen down through this structure even when no oxygen was present in the soil and water of the root zone.

Vlasis and Davis (52) obtained evidence that the rice plant is able to obtain oxygen from the atmosphere. They grew both excised and intact tomato, barley, and rice seedlings in nutrient solutions and measured their bromide uptake. The excised roots of all of the plants behaved similarly in their absorption of bromide when the concentration of oxygen in the aerating gas of the nutrient solution was 0.2 per cent. The intact plants responded very differently in their bromide absorption under the same conditions with the tomato absorbing very little bromide, the barley absorbing a considerable amount, and the rice plant absorbing almost as much as when aerated with air. This indicates that the excised roots of all three of the species were equally affected by a lack of oxygen. When the entire plant was used, however, barley, and especially rice, were able to obtain enough oxygen for accumulation of salt. The oxygen may have come from the atmosphere by diffusion through the plant.

Harrison and Aiyer (18) stated that the oxygen used by rice is obtained by movement through the flood water to the roots of the rice plants. They maintained that this oxygen

is provided by algae at the surface of the flood water. These algae use the carbon dioxide produced by symbiotic organisms from the methane and hydrogen gas given off from the soil. The oxygen produced by the algae moves downward through the flood water to the rice roots.

The diffusion of the oxygen present in the flood water was measured by Subrahmanyam (48). He added oxygen-saturated flood water to a soil and measured the concentration of oxygen as a function of depth. The oxygen concentration decreased slowly with depth for the first 3 inches from a saturation concentration of about 8 parts per million. Below 3 inches the oxygen content fell to about 5 parts per million, at which concentration it remained fairly constant through the remaining depth of the flood water. This absorption of oxygen by the rice soil was thought by Subrahmanyam to be non-biological in nature since he could detect no corresponding evolution of carbon dioxide from the soil. Schlapfer, et al. (44) noted that in a stagnant column of water the absorption and movement of oxygen was due mainly to the effects of convection. At the air-water interface, however, diffusion accounted for a large part of the absorption of oxygen by the water.

A large amount of controversial data exists on the effect of oxygen and the oxidation level of the soil on the nitrogen transformations in the soil. Russell (41) states that the oxidized forms of many systems in the soil, such as nitrates,

ferric iron, and sulfates, can be used by soil microorganisms as substitutes for oxygen in their respiration processes. Depending on the bacteria concerned and the conditions under which they are working, the nitrates in a soil can be reduced to nitrites, oxides of nitrogen, nitrogen gas or ammonia.

Broadbent and Stojanovic (8) studied the effect of the partial pressure of oxygen on the transformations of ammonium and nitrate nitrogen in a Dunkirk silty clay loam and a Mardin silt loam. There was very little nitrate reduced all the way to ammonia at any oxygen concentration. Only 5 per cent of the nitrate was reduced to ammonia even under anaerobic conditions. Considerable reduction and loss of nitrate occurred at all levels of oxygen from 0.1 per cent to 19 per cent. The denitrification of the nitrate nitrogen was inversely related to the oxygen supply since the largest losses of nitrate occurred at the lowest oxygen levels. When 2 per cent of corn leaves was added to the soil, the loss of nitrogen from the added nitrate was as high as 61 per cent at an oxygen level of 0.1 per cent.

The loss of elemental nitrogen from a Godarvus loam from Maryland has been studied by Jones (22). Using 200 parts per million of nitrogen in the form of nitrate, nitrite, and ammonia, Jones found, under anaerobic conditions which were produced by a vacuum, that as much as 80 per cent of the nitrate nitrogen was lost as elemental nitrogen. The same loss occurred when nitrites were used. Tracer techniques utilizing N^{15} showed

that no loss of ammonia as elemental nitrogen occurred when ammonia was added to the soil.

The rate of nitrification as influenced by the oxygen content of the soil air has been studied by Amer and Bartholomew (1). They found the rate of nitrification increased as the oxygen concentration of the soil air was increased. The greatest increase in nitrification was obtained from the first increment of oxygen with the increases becoming less as additional increments of oxygen were used. Dolarenko (27) also measured the increase in nitrate formation with oxygen content of the soil. An increase in the oxygen content of the soil air from 5.4 per cent to 19.4 per cent produced an increase in nitrate nitrogen content from 9 parts per million to 34 parts per million.

Sacks and Barker (43) have investigated the effect of oxygen on nitrate-reducing and nitrite-reducing enzymes. They noted that the presence of oxygen suppressed the formation of these enzymes. Oxygen also decreased the rate of reduction of nitrates and nitrites even when these enzymes were present. Over short periods of time this inhibition of nitrate and nitrite reduction was found to be reversible.

Bain and Chapman (4) tested the hypothesis that the nitrate form of nitrogen is more beneficial than the ammonium form in waterlogged soils due to the fact that the nitrate ion contains oxygen. They added both nitrate and ammonium nitrogen to waterlogged soils in which avocado and grapefruit

seedlings were growing. Less benefit was obtained from nitrate nitrogen than from ammonium nitrogen. This was explained as being due to the better competition of the denitrifying organisms for the added nitrates as compared to the absorption by plants. Since the nitrates were not absorbed, no extra oxygen was provided to the seedlings. Further, these workers contend that since the accumulation of nitrate nitrogen is also dependent on oxygen being present, the absorption ceased in the absence of oxygen.

Gillispie (16) noted that when soils were flooded they became highly reducing according to the potential on a platinum electrode. Upon being waterlogged the pH as measured by a hydrogen electrode showed a decrease in acidity. Not much significance was attached to this because of the possibility of the electrode being poisoned by some of the reduced substances in the soil. The influence of flooding on the nitrogen status of a soil has been noted by numerous workers. Nagaoka (33) found that some of the nitrate nitrogen added to flooded rice soils was lost. Harrison and Aiyer (17) discovered that drying a swamp soil caused a rapid increase in the rate of nitrification. Subrahmanyam (48) noted that waterlogging an Indian soil and a soil from the Rothamsted Experimental Station caused a distinct increase in the concentration of ammonia but only a slight decrease in the nitrate concentration. He could detect no loss or gain of total nitrogen from waterlogging. Allowing the waterlogged soil to dry

caused a rapid decrease in the ammonia content, although very little was lost by volatilization. The rapid increase in nitrate nitrogen which also occurred indicated that the ammonia was oxidized to nitrates after oxygen re-entered the soil. Bhuiyan (7) studied the distribution of different nitrogen fractions in flooded paddies during the growth period of rice. After flooding the ammonia content rose rapidly while nitrates disappeared from the soil. When the flood water was taken off the field the ammonia disappeared and the nitrate form reappeared.

Pearsall (35) and Pearsall and Mortimer (36) made oxidation-reduction potential, or E_H , measurements corrected to pH 5 on a large number of English soils. They noted a qualitative relationship between the E_H and the oxidized and reduced forms of several oxidation-reduction systems in the soil. At an E_H of 320 millivolts or less, the ammonium form of nitrogen as well as the ferrous form of iron and the sulfide form of sulfur predominated over their respective oxidized forms: nitrate nitrogen, ferric iron, and sulfate sulfur. As a general rule, soils which had been flooded gave an E_H of 200 millivolts or less, while unflooded soils almost always had an E_H of 380 millivolts or higher. Working with natural waters, Pearsall and Mortimer demonstrated the effect of aeration on the E_H . When air was bubbled through the system the E_H rose immediately. Upon displacing the oxygen with nitrogen the E_H decreased. No indication of the oxygen

status of these waters could be obtained, however, from the E_h value alone.

The effects of waterlogging and the decomposition of organic matter on the oxidation level of typical rice soils has been studied by Sturgis (47). The development of a low E_h in waterlogged soils was largely dependent on the decomposition of fresh organic matter. The E_h of a Crowley silt loam which had just previously been cropped with rice decreased from 570 millivolts to a constant value of 540 millivolts upon flooding. The E_h of the same soil which had received 0.5 per cent soybean hay dropped from 670 millivolts to a constant level of 90 millivolts. Sturgis found that the ammonium nitrogen content of an untreated Sharkey clay loam which had been flooded for three months was 47.7 parts per million compared to 7.9 parts per million in the same soil kept at optimum moisture. No nitrate nitrogen was present in the flooded soil compared to 62 parts per million in the soil not flooded. The E_h after three months was 70 millivolts for the flooded soil and 490 millivolts for the soil kept at optimum moisture. Reed and Sturgis (38, 39) found that flooding a Crowley silt loam increased the pH and the concentrations of soluble magnesium, sodium, iron, and silica. The nitrates in the soil decreased rapidly after flooding and disappeared completely before harvesting the rice. Flooding also caused a decrease in the concentration of ammonia although the ammonia did not disappear

as rapidly as did the nitrates. Nitrites were not found in amounts larger than 3 parts per million.

Willis and Sturgis (55) investigated the loss of nitrogen from an uncropped flooded soil as affected by changes in temperature and reaction. They found that at a high moisture content a large amount of ammonia was lost from the soil when incubated at 100° F. An increased pH induced by liming increased the loss of ammonia from the soil. This loss of ammonia might not have been as large if rice had been present in the cultures. A lesser loss of ammonia due to the presence of the plants has been demonstrated by Willis and Green (55). They detected ammonium nitrogen in the flood water of both cropped and uncropped Crowley silt loam and Lake Charles clay loam soon after flooding. The ammonia content decreased rapidly in the cultures containing plants but remained fairly constant in the unplanted cultures. The decrease in ammonia concentration in the pots containing rice was most probably the result of ammonia absorption by the plants. Large losses of nitrogen to the atmosphere occurred in the highly fertilized series which did not contain rice, but such losses did not occur in the cropped series. Steenbjerg (46) also noted the relationship between pH and loss of ammonia. He found that no ammonia was lost from the application of ammonium sulfate to an arable soil at pH 6. When the pH was increased the ammonia loss became considerable. The loss was always high when the soil contained considerable calcium carbonate. Little

loss occurred when the ammonium sulfate was applied 2.5 inches into the soil.

The relatively poor response made by rice to fertilizer nitrogen has led to a large number of studies on the nitrogen nutrition of rice. Many of the investigations performed in soil cultures have been complicated by the changes in the forms and amounts of nitrogen present during the experimental period. On the other hand, the results obtained from experiments carried out with nutrient solutions may not be strictly applicable to soils. Some of the difficulties involved in extending the results obtained using nutrient solutions to soils have been discussed by Hoagland and Martin (20).

Most of the experimental work on the effect of different forms of nitrogen on the growth of rice has shown that the ammonium form is superior to the nitrate form. The increased value from ammonium nitrogen is usually attributed either to its greater stability in the flooded soil system or to its superiority to nitrate nitrogen as a nutrient ion for rice. Kellner (26) in 1884 found that rice in paddy soils responded better to ammonium nitrogen than to nitrate nitrogen in the early stages of its growth. Later in the growth period, however, nitrates were the most effective. Kellner also noted that nitrates were formed very slowly in paddy soils while ammonia was formed in large quantities. Working in Hawaii, Kelley (25) noted that nitrate nitrogen was a poor source of nitrogen for rice. In sand cultures poor

plants were produced from nitrate nitrogen while ammonium nitrogen produced healthy and vigorous plants. Bartholomew (5) recommended the use of ammonium forms of nitrogen, although in his experiment the yields from treatments receiving sodium nitrate were only about 10 per cent less than that from treatments receiving ammonium nitrogen. He justified this recommendation of the ammonium form over the nitrate form of nitrogen by pointing out that, in addition to the slightly higher yield from the use of ammonium nitrogen, this form was not as likely as nitrate nitrogen to be lost from the soil through denitrification.

Gericks (14) grew rice to maturity in nutrient solutions with nitrates as the only source of nitrogen. Good growth was obtained but no treatments involving ammonium nitrogen were included for comparison. Ammonium sulfate was superior to calcium nitrate for rice in a nutrient solution experiment conducted by Kapp (24). Not only was a higher yield produced from the ammonium nitrogen than from the nitrate nitrogen, but the percent nitrogen in the plants was higher when ammonium nitrogen was used. Ammonium sulfate also proved superior to sodium nitrate for rice on Crowley silt loam. The rice plants from the ammonia treatments did not contain the largest percentage of nitrogen in this case.

Walker and Sturgis (53) found at four locations in the Coastal Prairie soils of Louisiana that the application of 32 pounds per acre of nitrogen from ammonium sulfate as a

topdressing with no other fertilizer increased the yield of rice 11.6 bushels per acre. An increase of 11.3 bushels per acre was obtained from ammonium nitrate and no increase was obtained from sodium nitrate. When the rice was topdressed with the same three materials plus 300 pounds per acre of 3-9-5 applied at the time of planting, the increases in yield were: 12.1 bushels per acre from ammonium sulfate, 6.2 bushels per acre from ammonium nitrate, and 10.6 bushels per acre from sodium nitrate. It appears that when applied alone as a topdressing, ammonium sulfate was superior to sodium nitrate in these experiments, but when the same nitrogen sources were used in addition to 300 pounds per acre of 3-9-5 applied at the time of planting, the two were equal in effectiveness.

Reporting on the results of rice fertilizer experiments in Arkansas from 1946 through 1951, Beacher (5) found that ammonium sulfate and the synthetic organic forms of nitrogen were superior to nitrates as fertilizers for rice. Jones (23) found that the use of 30 pounds of nitrogen as ammonium sulfate increased the yield of rice 15 per cent on Stockton clay adobe in California for the five year period 1938-1942. This was compared to 17 per cent increase from uramon and a 4 per cent increase from calcium nitrate. The application of 1.5 tons per acre of rice straw every other year caused a decrease in yield of 8 per cent under the yield of the check. When the straw was applied in combination with 30 pounds of nitrogen as ammonium sulfate the yield was increased 7 per cent.

The oxidized form of some nutrient ions may be able to provide the oxygen necessary for the absorption of ions by plants. Arnon (3) found that the lack of oxygen in cultures containing barley had a detrimental effect on the barley which received ammonium nitrogen, while no adverse effect was observed from the lack of oxygen in similar cultures containing nitrate nitrogen. Arnon postulated that the oxygen in the nitrate nitrogen may have been available to the plant for respiration. Warburg and Negelein (54) discovered that the respiration quotient of Chlorella was increased from 1.00 - 1.04 to 1.3 - 2.0 following the absorption of nitrates by the cell. The extra oxygen necessary to raise the respiratory quotient came from the nitrates reduced after entering the cell. There was an increase in nitrites in the Chlorella when supplied with nitrates and subjected to anaerobic conditions. These workers also noted that the reduction of nitrates was inhibited by the presence of oxygen.

In a very comprehensive study, Shive (45) sought to determine the effect of oxygen on the absorption and accumulation of nitrogen by soybeans. He found that the highest rate of absorption of nitrate nitrogen occurred at low levels of oxygen. As the level of oxygen increased the rate of nitrate intake decreased. The highest rate of absorption of ammonium nitrogen took place at the highest levels of oxygen. The accumulation of nitrates within the roots of the plants was greatest at high oxygen concentrations of the nutrient solution.

This was probably because the nitrates in the low oxygen treatments were being reduced with the release of oxygen for root respiration. Since this oxygen was not needed in the cultures receiving oxygen, the nitrates tended to accumulate. A further indication that the nitrate nitrogen was reduced in order that its oxygen might be released was the fact that at the low oxygen levels, the accumulation of reduced forms of nitrogen was highest.

Gilbert and Shive (15) found that the use of nitrate nitrogen in tomatoes, oats, and soybeans in nutrient solutions resulted in a higher rate of carbon dioxide production than was obtained when these same plants were grown in nutrient solutions containing ammonium nitrogen or no nitrogen. The extra respiratory carbon dioxide produced by the plants receiving nitrate nitrogen was greatest at low oxygen concentrations and was very closely related to nitrate absorption and nitrate reduction. The production of this extra respiratory carbon dioxide appeared to be directly associated with the release of oxygen by nitrate reduction and its utilization in respiration. These workers also found that the maximum absorption and maximum reduction of nitrate ions occurred at the lowest oxygen tension of the nutrient cultures.

In contrast to the findings of Shive, and Gilbert and Shive, Nance (34) found that oxygen in the nutrient solution enhanced the absorption of nitrates by excised wheat roots. Placing excised wheat roots in a solution which contained

0.002 molar potassium nitrate resulted in the absorption of 35.2 micromoles of nitrates by roots aerated with air while roots aerated with nitrogen absorbed only 3.7 micromoles of nitrates. The assimilation of the absorbed nitrates, however, was much greater on a percentage basis in the treatment receiving no oxygen. The roots assimilated 76 per cent of the absorbed nitrates compared to an assimilation of 32 per cent in the solution containing oxygen.

EXPERIMENTAL

Materials and Methods

This study consisted of two greenhouse experiments and several laboratory experiments conducted in the laboratory and greenhouse at Louisiana State University, Baton Rouge, and a field experiment carried out at the Rice Experiment Station, Crowley, Louisiana.

The first greenhouse experiment was designed to determine the effects of different oxidation levels of a flooded rice soil on the rice plant's response to two different forms of nitrogen and two different levels of potash. Three treatment factors were used in this experiment.

One treatment factor consisted of various oxidation levels of the soil and flood water. The treatments were:

- a. Flooding the soil to a depth of 5 inches plus bubbling oxygen continuously through the soil and flood water.
- b. Flooding the soil to a depth of 5 inches plus a hydrolyzed polyacrylonitrile soil conditioner added to the soil at the rate of 0.05 per cent.
- c. Flooding the soil to a depth of 5 inches.

In addition to these three treatments a treatment in which the soil was kept at 15 per cent moisture was included, but the growth of rice obtained was so poor that this treatment is not included in the results.

The second treatment factor used was different sources of nitrogen added to the soil. The different sources of nitrogen were:

a. Sodium nitrate at the rate of 40 pounds of nitrogen per acre before transplanting and 40 pounds per acre as a topdressing 6 weeks after transplanting.

b. Ammonium sulfate at the rate of 40 pounds of nitrogen per acre at time of transplanting and 40 pounds per acre as a topdressing 6 weeks after transplanting.

Two different levels of potash were used as the third treatment factor. These two levels were:

a. No potash.

b. Potassium chloride at the rate of 80 pounds of potash per acre.

The above three treatment factors were combined factorially and three replications of each treatment combination were used. In addition to the factorial experiment described above, a check which received no addition of fertilizer was included. Phosphorus at the rate of 80 pounds per acre of phosphorus pentoxide as monocalcium phosphate was added to all of the treatments in the experiment except the check. Crowley silt loam from the Old Rice Experiment Station at Crowley, Louisiana was used in this experiment. The different fertilizer treatments were mixed with 12 kilograms of air-dry sieved soil and transferred to 5 gallon earthenware pots. The soil in the pots was saturated with water and 30 rice seedlings which had

been grown in greenhouse flats were transplanted to each pot a week after the fertilizer had been added. As the rice plants grew, the level of the flood water was raised gradually to 5 inches and was maintained at this level.

Ten rice plants from each pot were harvested at three different times during the growing season. Only the above-ground portions of the rice plants were harvested. The rice plants were dried in a draft oven at 60° C., weighed, and analysed for potassium and nitrogen. Potassium was determined by the method of Teth et al. (50) with slight modifications. The Kjeldahl method was used for the determination of nitrogen in the plant.

Nitrate nitrogen, ammonium nitrogen, and dissolved oxygen in the soil solution of the root zone were measured in this experiment. The samples were taken by introducing a Pasteur-Chamberland filter filled with distilled water into the root zone for three days. A preliminary experiment showed that two days or less was sufficient time for the solution within the cell to reach equilibrium with that in the root zone. The phenoldisulfonic acid method was used for the determination of nitrates and ammonium nitrogen was determined by nesslerization. The solutions were centrifuged to insure a clear solution and the color intensities measured with an electrophotometer.

In the early part of this study, dissolved oxygen was determined by the Rideal-Stewart modification of the Winkler method (2). This method, which calls for approximately 100

milliliter of sample, was further modified in order to determine the oxygen in a 10 milliliter sample. The samples were drawn into a 25 milliliter hypodermic syringe from the sampling cell with care being taken that air was excluded from the solution. The concentrations of the reagents were adjusted so that 1 milliliter of each reagent was used. These reagents were stored in serum bottles with rubber caps and were introduced into the solution by puncturing the cap of the serum bottle with the hypodermic needle and withdrawing 1 milliliter of the reagent. In this manner oxygen from the air was prevented from contaminating the sample.

Later in this study dissolved oxygen was determined polarographically with a Sargent-Heyrovsky photographic recording polarograph. The soil solution was transferred from the Pasteur-Chamberland filter to an electrolysis vessel and analyzed for oxygen. Methyl red was used as a maximum suppressor although Evans and Scott (12) have shown that no oxygen maximum occurs in water-saturated soils.

The oxidation-reduction potential, or E_h , of the soil was measured during the course of the experiment. This potential was determined on a platinum electrode utilizing a saturated calomel half cell as a reference electrode. Both Beckman Model M and Model H-2 pH meters were used to measure the potential between the calomel half cell and the platinum electrode inserted in the soil. The platinum electrodes were constructed by sealing a 1-centimeter piece of 18 gauge

platinum wire in the tip of a piece of pyrex glass tubing which had the same coefficient of thermal expansion as the platinum. A few drops of mercury on the inside of the glass tubing was used as a contact between the platinum and the copper wire leading to the potentiometer. The electrodes were cleaned by inserting them in boiling chromic acid for several minutes, washing them thoroughly with distilled water, and soaking them in distilled water until used. Bright platinum electrodes were selected instead of platinized electrodes because there was a greater possibility of the platinized electrodes being poisoned at low E_H values. The E_H measurements were made in situ in order to escape the complications arising from taking the samples to the laboratory for the measurements. The platinum electrode was inserted to a depth of approximately 3 inches into the soil and the calomel electrode was placed in the flood water to complete the cell. pH determinations were made with the same instruments that were utilized for the E_H measurements except that a glass electrode was substituted for the platinum electrode.

The second greenhouse experiment was conducted primarily to measure the effect of various oxidation levels of a flooded Crowley silt loam on the losses of ammonium nitrogen by volatilization from the soil and flood water. The treatments used in this experiment were:

- a. Flooding the rice soil to a depth of 5 inches plus bubbling oxygen continuously through the soil and flood water.

- b. Flooding the soil to a depth of 5 inches.
- c. Flooding the soil to a depth of 5 inches plus the addition of 0.2 per cent ground clover hay to the soil.

These treatments were combined factorially with the following two treatments:

- a. Sodium nitrate at the rate of 40 pounds of nitrogen per acre before transplanting and 40 pounds per acre as a topdressing 5 weeks after transplanting.
- b. Ammonium sulfate at the rate of 40 pounds of nitrogen per acre before transplanting and 40 pounds per acre as a topdressing 5 weeks after transplanting.

The six treatment combinations were run in duplicate. Crowley silt loam, to which 80 pounds per acre of phosphorus pentoxide as monocalcium phosphate and 80 pounds per acre of potash were added, was used in this study. Ten rice plants were transplanted to each pot and the water level raised gradually as the plants grew. The plants were harvested after seven weeks.

In this experiment the nitrate and ammonium nitrogen in the flood water was determined at intervals after the rice was topdressed. In order to retain the ammonia, the ammonia lost from the rice soil by volatilization was measured by forcing all of the air from the rice cultures through a 4 per cent solution of boric acid. Eight-liter pyrex percolation flasks were inverted over the two gallon pots and secured with tape. The air under the inverted flasks was swept out continuously by using a suction pump to pull the air from the culture through the boric acid. This created a slight pressure

deficit inside the culture which prevented any air from escaping except through the boric acid. The boric acid was changed at intervals and the amount of ammonia lost determined by titrating the ammonia held in the boric acid with hydrochloric acid.

The field experiment was designed to determine the effect of different oxidation levels of the soil on the rice plant's response to different forms of nitrogen added as topdressings. The sources of nitrogen used were: ammonium sulfate, sodium nitrate, ammonium nitrate, aqua ammonia, and urea. These were applied as topdressings in the flood water at the rate of 50 pounds of nitrogen per acre at two different times, 6 weeks after planting, and 9 weeks after planting. The treatments used to alter the oxidation level of the soil were:

- a. A hydrolyzed polyacrylonitrile soil conditioner added to the soil at the rate of 0.05 per cent.
- b. Rice straw added at the rate of 5000 pounds per acre.
- c. No treatment.

The ammonium sulfate and sodium nitrate treatments, the applications applied at 6 and 9 weeks, and the three treatments used to modify the oxidation level of the soil were all combined factorially to give twelve treatment combinations. In addition to the above experimental design, the other nitrogen sources, aqua ammonia, ammonium nitrate, and urea, were combined factorially with the 6 and 9 week applications

and these six treatment combinations used without additions of soil conditioner or rice straw. All of the treatment combinations in this experiment were replicated three times. The plot size was 10 feet square with each plot being surrounded by a levee. The soil conditioner and rice straw were well disked into the soil at optimum moisture. Three hundred pounds per acre of a 3-12-12 fertilizer was applied at the time of planting. Zenith rice was planted April 8, 1953 and the rice was flooded May 6, 1953. The plots were top-dressed by broadcasting the fertilizer uniformly over the flood water of each plot at two different dates, 6 weeks and 9 weeks after planting.

Six days after the topdressing, a sample of rice plants from each plot was taken for analysis of total nitrogen. The E_g of the soil was measured in the same manner as in the greenhouse experiments. Dissolved oxygen was measured by the polarographic method used in the greenhouse work. The rice was harvested August 11, 1953.

The nutrient solution experiment was designed to measure the influence of oxygen in the root substrate on the uptake of ammonium and nitrate nitrogen by rice plants. A modified Knop solution was used in which ammonium sulfate and sodium nitrate were the sources of nitrogen. Each of these two nitrogen sources was used at two different levels of oxygen in the nutrient solution. The two oxygen levels were obtained by saturating one series of nutrient solutions

with oxygen and saturating the other series with nitrogen. Polarograms of the two nutrient solutions are shown in figure 1. Oxygen was not detectable in the nitrogen-saturated solution, whereas the oxygen concentration of the oxygen-saturated solution varied around 30 parts per million. One-quart Mason jars were used for growing the plants with 4 rice plants being used per jar. Each treatment was duplicated. After the experiment was started, the nitrate and ammonium nitrogen of the nutrient solutions were measured at 2 to 3 day intervals by the methods listed above. The duration of the experiment was not sufficient for differences in the growth of the rice plants to be manifested.

In order to collect some additional information on the influence of oxygen on the absorption of nitrate and ammonium nitrogen, a laboratory experiment was conducted in which rice was grown on a Crowley silt loam under two different oxygen levels. One hundred and fifty grams of air-dry soil was placed in 250 milliliter wide-mouth Erlenmeyer flasks and flooded. Each flask received the equivalent of 100 pounds per acre of nitrogen, phosphorus pentoxide, and potash. Two sources of nitrogen were used, ammonium sulfate and sodium nitrate. One rice plant was transplanted to each flask by inserting the plant through a cork stopper and fixing the stopper in the flask. The plants were transferred to the flasks 2 weeks before the nitrogen was added. One oxygen level was obtained by bubbling air through the

Figure 1.-Polarograms of oxygen-saturated and nitrogen-saturated nutrient solutions. 1 part per million oxygen = 2.8 millimeters deflection.

soil and flood water of the culture. Care was taken that the oxygen was distributed throughout the soil. The other oxygen level was obtained by restricting the exchange of gases between the flask and the atmosphere. This was accomplished by leaving only a small opening in the cork. Measurements of oxygen showed that there were 6 parts per million of oxygen in the root zone of the aerated cultures and no oxygen in the unaerated cultures. Six replications of each of the four treatment combinations were made. The rice plants were harvested at 2 to 4 day intervals, weighed, and analyzed for total nitrogen. The nitrate and ammonium nitrogen in the flood water and soil were determined by transferring both the soil and flood water to a Buechner funnel and leaching with a normal sodium chloride solution. The leachate was then analyzed for nitrate and ammonium nitrogen (57).

The extent to which a reducible or oxidizable substance will be reduced or oxidized by a biological system cannot be determined by the E_h measurement alone, since this measurement indicates only the intensity of reduction or oxidation. The degree to which a given amount of an oxidized substance will be reduced in such a system depends on the difference between the potentials of the oxidized substance and the medium, and also on the kinds and amounts of reduced substances that are in the biological system. The use of the oxidation-reduction potential alone in characterizing soil aeration has met with only limited success in predicting

the growth of plants under different soil conditions. It is possible that a measurement which would give the rate of reduction of which a soil is capable may yield a better picture of the oxidation-reduction level of the system than the measurement of the E_H alone.

A laboratory experiment was conducted in order to develop a method by which the rate of reduction of oxygen by a soil suspension could be measured. Several methods were tried before one was selected as being the most satisfactory. The procedure adopted consisted of saturating a soil suspension with oxygen and measuring the oxygen concentration of the suspension as a function of time in a closed system. The soil suspension was saturated by aerating with oxygen as it was being stirred with a high-speed mechanical stirrer. The oxygen-saturated suspension was then transferred to a 250 milliliter wide-mouth Erlenmeyer flask which was then completely filled with the suspension. The flask was immediately stoppered to prevent the entry of air and was then placed on a magnetic stirrer which kept the suspension agitated during the course of the experiment. The flask contained approximately 75 grams of soil. A dropping mercury electrode, a platinum electrode, and a salt bridge connected to a calomel half cell were enclosed in the stopper. The first measurements were made as rapidly as possible after the soil had been saturated with oxygen. Each time at which an oxygen measurement was made, the E_H of the system was also

recorded in order to determine if any relationship existed between the oxygen concentration and the E_H . Measurements made by this method have been employed in the interpretation of results from this study.

Results and Discussion

Data on the growth of rice obtained from the first greenhouse experiment are shown in table 1. Ammonium nitrogen produced a more rapid growth of rice during most of the growing season. This increased growth was evident only at the first harvest or in the first part of the growing season, as there were no significant differences between the average yields from the nitrate nitrogen treatments and the ammonium nitrogen treatments at the last harvest. The data in tables 2 and 3 indicate that the increased early yields from the pots receiving ammonium nitrogen may have been due to a greater absorption of the ammonium form of nitrogen. At the first harvest, the rice from the ammonium nitrogen treatments contained considerably more nitrogen than the rice from the nitrate treatments. There was a high initial concentration of ammonium nitrogen in the root zone of the rice from the ammonium nitrogen treatments, table 2. At the second harvest, the yield was still greater from the ammonium nitrogen treatments, although the per cent of nitrogen was higher in the nitrate nitrogen treatments. This does not necessarily indicate that the higher nitrogen content in the rice at the second harvest from the nitrate treatments was due to the absorption of the added nitrates because the nitrates had disappeared from the soil by this time. Ammonium nitrogen formed by the

Table 1.-Yield of rice in grams per pot at three dates of harvest as influenced by source of nitrogen, oxidation treatments, and addition of potash in a greenhouse experiment on Crowley silt loam.

Oxidation treatment	Source of N	No potash			Potash			Average		
		42 da.	58 da.	78 da.	42 da.	58 da.	78 da.	42 da.	58 da.	78 da.
Soil conditioner	NO ₃	2.04	5.97	21.8	2.25	5.90	22.1	2.14	5.94	21.9
	NH ₃	3.52	10.3	23.0	2.71	7.55	29.9	3.12	8.92	26.4
Oxygen	NO ₃	2.97	9.17	31.7	3.54	7.39	29.6	3.26	8.28	30.7
	NH ₃	3.74	9.36	31.6	3.93	11.1	27.8	3.84	10.2	29.7
No treatment	NO ₃	2.42	7.77	28.7	2.54	7.72	25.8	2.48	7.74	27.2
	NH ₃	3.62	9.77	28.9	3.81	9.85	24.4	3.72	9.81	26.7
Average	NO ₃	2.48	7.64	27.4	2.78	7.00	25.8	2.63	7.32	26.6
	NH ₃	3.63	9.81	27.8	3.48	9.48	27.4	3.56	9.65	27.6
Check		2.50	8.12	16.9						

	<u>42 days</u>	<u>58 days</u>	<u>78 days</u>
LSD (0.05) between any two yields	0.43	1.50	3.94
LSD (0.05) between averages of N treatments	0.25	0.86	2.26
LSD (0.05) between averages of nitrogen treatments at one oxidation treatment	0.35	1.21	3.20

Table 2.-The concentration of nitrate and ammonium nitrogen in parts per million in the soil solution of the root zone of rice in a greenhouse experiment on Crowley silt loam.

Source of nitrogen		Days after flooding				
		6	15	26	37	48
Ammonium sulfate	NO ₃ -N	0.18	0.20	0.10	0.12	0.08
	NH ₃ -N	2.92	2.67	2.88	1.72	1.92
Sodium nitrate	NO ₃ -N	-	2.28	0.21	0.11	0.09
	NH ₃ -N	2.12	1.92	1.65	1.33	1.25
No nitrogen	NO ₃ -N	0.20	0.19	0.11	0.12	0.10
	NH ₃ -N	3.60	3.05	2.42	1.30	1.02

Table 3.-Per cent nitrogen in rice plants at three dates of harvest as influenced by source of nitrogen and oxidation treatment in a greenhouse experiment on Crowley silt loam.

Oxidation treatment	Source of N	42 days	58 days	78 days
Soil Conditioner	NO₃	1.49	1.61	1.31
	NH₃	1.64	1.32	1.08
Oxygen	NO₃	1.44	1.72	1.35
	NH₃	1.75	1.38	1.19
No treatment	NO₃	1.47	1.22	1.36
	NH₃	1.45	1.39	1.18
Average	NO₃	1.47	1.52	1.34
	NH₃	1.61	1.36	1.15
Check		-	1.21	1.05

reduction of nitrate nitrogen was most likely the form of nitrogen being absorbed.

Although previous work has shown that rice can produce good growth without oxygen in the root zone, a significant increase in the yield of rice was obtained through the application of oxygen to the soil. This increase in growth was obtained at all three harvests. The added oxygen may have been of some metabolic value to the rice plant by aiding the absorption of nutrient ions. Another possible explanation for the response to added oxygen is that oxygen may have prevented the accumulation of reduced products of microbial and chemical action, and thereby it may have aided the growth of the rice plants indirectly. There was no indication that the added oxygen prevented the reduction of nitrates or aided the oxidation of ammonium nitrogen in the soil.

The application of the soil conditioner to the rice soil resulted in a decrease in the growth of rice. This decrease was very likely due to the increased growth of weeds in the pots receiving this treatment. This greater growth of weeds appeared to have been caused by the clearing of the flood water by the soil conditioner, thereby allowing more light to penetrate to the soil surface than on the other treatments and thus stimulating the growth of water weeds.

No increase in the growth of rice was obtained from the addition of 80 pounds of potash per acre, table 1. The

potassium content of the above-ground portion of the rice plant was markedly affected by the addition of potash to the soil, table 4. In every treatment receiving potash, the potassium content of the rice plant was higher than in the corresponding treatment without potash. For the first harvest where potash was added, the potassium content of the rice from the treatment receiving oxygen was slightly higher than the potassium content of the rice from the no-oxygen treatments. The potassium concentration in the plants was highest during the early growth of the rice. As the season progressed, the potassium content of the rice decreased. At the second harvest, or rice harvested at 58 days after planting, the potassium content was higher in the rice from the ammonium than from the nitrate nitrogen treatments. There was no difference between the potassium contents of the rice from the nitrate and ammonium treatments for the other two harvests.

The oxidation-reduction potential, or E_H , of the soil in all of the treatments decreased within 3 weeks after flooding from approximately 400 millivolts to slightly less than 100 millivolts. There was no further decrease in the E_H of the different treatments during the course of the experiment. In all cases, the E_H of the flood water was higher than that of the soil below it.

The dissolved oxygen in the soil disappeared rapidly after flooding. The free oxygen usually disappeared from

Table 4.-Potassium content of rice in milliequivalents per 100 grams of oven-dry material at three dates of harvest as influenced by source of nitrogen, oxidation treatments, and addition of potash in a greenhouse experiment on Crowley silt loam.

Oxidation treatment	Source of N	No potash			Potash			Average		
		42 da.	58 da.	78 da.	42 da.	58 da.	78 da.	42 da.	58 da.	78 da.
Soil conditioner	NO ₃	51	37	30	80	38	38	66	39	34
	NH ₃	46	45	27	72	66	37	59	56	32
Oxygen	NO ₂	56	34	24	82	58	32	62	36	28
	NH ₃	56	37	21	81	42	24	69	43	26
No treatment	NO ₃	56	30	24	67	48	36	62	39	31
	NH ₃	58	38	24	78	53	35	68	46	30
Average	NO ₃	54	34	26	76	48	36	65	41	31
	NH ₃	53	40	24	77	56	35	65	48	30
Check		54	45	38						

the root zone within two days. The distribution of dissolved oxygen in the soil and flood water is shown in figure 2. At the surface of the flood water in the pots which did not receive oxygen, the oxygen concentration was approximately 8.2 parts per million. This is the oxygen saturation concentration in water under the partial pressure of oxygen in the atmosphere at 26° C., which was the approximate temperature at which these measurements were taken. One centimeter under the surface of the water the oxygen concentration was 6.1 parts per million. The oxygen concentration remained fairly constant below the 1-centimeter depth throughout the rest of the flood water but decreased very rapidly with depth after the surface of the soil was reached. The oxygen concentration of the soil was nil less than one centimeter below the soil-water interface. There was, therefore, a considerable oxygen gradient between the flood water and the uppermost layer of soil. The influence of the oxygen reaching the soil by diffusion was therefore restricted to the roots concentrated immediately in the soil-water interface. The oxygen moving downward into the soil was used rapidly as it reached the soil-water interface. The rate at which oxygen is consumed by a soil depends greatly on the treatment to which the soil has been subjected. This is discussed more completely in a later part of this study. As shown in figure 11, the more reduced the soil system, the more rapid is the consumption of oxygen.

**THE CHANGE IN OXYGEN CONCENTRATION
AS A FUNCTION OF DEPTH**

Figure 2.-The distribution of oxygen in the soil and flood water of a 3-gallon pot containing 7-week-old rice.

In the pots receiving oxygen, the oxygen concentration of the flood water varied between 15 and 35 parts per million depending on how fast the oxygen was bubbled through. No oxygen gradient existed within the flood water due to the turbulence created by the oxygen bubbles. In the treatments being aerated, the oxygen concentration in the water directly above the soil was considerably higher than in the treatments receiving no oxygen. There was, consequently, a greater oxygen gradient across the soil-water interface of the aerated treatments which caused more oxygen to move downward into the root zone. As in the treatments which did not receive oxygen, no oxygen was present in the soil 1 centimeter below the soil-water interface in the treatments which received oxygen.

In the second greenhouse experiment, which was designed to measure the effects of different oxidation levels on the loss of nitrogen and the growth of rice, no increase in the growth of rice was obtained from the use of oxygen, table 5. The yield from the pots receiving ammonium nitrogen was considerably higher than the yield from pots receiving nitrate nitrogen. The nitrogen content of the plants from all of the treatments was higher in this experiment than in the first greenhouse experiment. The growth of rice in this second experiment was noticeably poorer than that obtained in the first experiment, which may have been responsible for the higher nitrogen content of the rice in the second experiment. There was no difference in the nitrogen content of the

Table 5.--Yield in grams per pot and per cent nitrogen of rice plants as influenced by source of nitrogen and oxidation treatment in a greenhouse experiment on Crowley silt loam.

Oxidation treatment	Source of N					
	NO ₃		NH ₃		Average	
	Yield	% N	Yield	% N	Yield	% N
Oxygen	4.05	2.74	7.39	2.48	5.72	2.61
Organic matter	3.75	2.83	6.50	3.13	5.13	2.98
No treatment	5.12	2.58	6.27	2.59	5.70	2.59

treatments receiving oxygen and those not receiving oxygen. The addition of 0.2 per cent clover hay to the soil caused an increase in the nitrogen content of the rice plants. This was probably due to the nitrogen added in the clover hay.

The E_H of the soil decreased rapidly after flooding, figure 3. There was a constant rate of decrease of the E_H after flooding in the treatments which did not receive organic matter. The E_H of the organic matter treatments fell rapidly after flooding but leveled off to a value of less than 100 millivolts. Concurrent with the most rapid rate of decrease of the E_H in the treatments receiving organic matter was the very poor growth made by the rice plants in those pots. This may have been due to the intensity of the reduction processes going on in the soil, or to the toxicity caused by the accumulation of reduced products and carbon dioxide from these processes.

The pH of the soil immediately after flooding was 6.0 for all of the treatments. The pH rose gradually in all of the treatments during the course of the experiment. The highest pH in the treatments which did not receive organic matter was 7.0, as compared to a high of 7.7 in the clover hay treatment. This difference in pH was probably due to the difference in the E_H of the two treatments. There is an inverse relationship between pH and E_H of an oxidation-reduction system. The difference of 0.7 pH unit would only account for a difference in E_H of 42 millivolts at 30° C.;

Figure 3.-The effect of flooding and organic matter on the E_H of a Crowley silt loam. Abscissa is time after flooding.

however, this increase in pH is considerably less than enough to account for the difference between E_H of the treatment receiving organic matter and the treatment receiving no organic matter, assuming that the E_H -pH relationship has the theoretical slope of 60 millivolts per pH unit.

A considerable amount of the nitrogen applied as a topdressing was lost by volatilization as ammonia from the treatments receiving organic matter, figure 4. This high loss of ammonia was probably caused in part by the reduced conditions of the soil in these treatments. It is likely that the increased pH in the treatments receiving organic matter as well as the high temperature of the rice flood water also increased the loss of ammonia by volatilization (56). Flood water temperatures as high as 100° F. were recorded. In addition, the extra nitrogen added in the organic matter probably also contributed to the increase in the loss of ammonia. A much smaller loss of ammonia took place from the treatments which received no organic matter, as is shown in figure 5. In both the organic and nonorganic treatments, the ammonia added as a topdressing of ammonium sulfate decreased rapidly in the flood water. As the ammonia concentration of the flood water decreased, the nitrate concentration increased, even under the reduced conditions in the organic matter treatments. This indicates that nitrification may have been taking place in the flood water, which is partially supported by the work of Mukerji and

Figure 4.-The loss of ammonium nitrogen by volatilization and the concentration of ammonium and nitrate nitrogen in the flood water after topdressing with ammonium sulfate. 0.2 per cent organic matter added.

Figure 5.-The loss of ammonium nitrogen by volatilization and the concentration of ammonium and nitrate nitrogen in the flood water after topdressing with ammonium sulfate. No organic matter added.

Vishnoi (32), who found aerobic bacteria present in submerged rice soils. In the organic matter treatment, figure 4, the nitrate concentration of the flood water rose to a maximum and then decreased as the ammonium nitrogen continued to disappear from the flood water.

In the field experiment conducted at the Rice Experiment Station at Crowley, Louisiana, no increase in yield was obtained from the use of any form of nitrogen, table 6. The very high yields obtained on the no-nitrogen treatment as well as on the treatments receiving nitrogen show that growth was not limited by a lack of nitrogen in this experiment. The farm on which this test was conducted has a history of high rice yields. Another factor which tended to raise the rice yield was the effect of the borders around each plot. There was a three-foot wide space inside each levee and around each rice plot. No rice was being grown in this area and consequently rice near the borders of the plots grew better than it would have grown under normal conditions. Even though no increase in yield resulted from the application of nitrogen, rice grown on treatments receiving nitrogen as a topdressing absorbed greater amounts of nitrogen than did rice on the no-nitrogen treatments, table 6. Except for the aqua ammonia treatment, the rice on the treatments which received an ammonium form of nitrogen absorbed a greater percentage of nitrogen than the rice on the nitrate treatments after both times of topdressings.

Table 6.-The yield of rice in barrels per acre and the per cent nitrogen in rice as affected by source of nitrogen, time of topdressing, and state of oxidation in a field experiment on Crowley silt loam.

Source of nitrogen	Time of topdressing weeks	No treatment		Soil conditioner		Rice straw		Average	
		Yield	% N	Yield	% N	Yield	% N	Yield	% N
No nitrogen	-	50.3	1.59	38.0	1.49	36.1	1.59	41.5	1.55
Sodium nitrate	6	39.2	1.91	44.9	2.36	38.5	2.42	40.9	2.23
	9	46.6	1.89	39.1	1.80	41.1	1.91	42.3	1.87
Ammonium sulfate	6	46.2	2.20	39.8	2.36	41.2	2.14	42.4	2.24
	9	44.7	2.12	37.4	2.03	39.0	2.04	40.4	2.06
Average		45.4	1.94	39.8	2.01	39.2	2.02		
Ammonium nitrate	6	50.0	2.41						
	9	45.1	1.99						
Ammonia	6	45.5	1.96						
	9	45.3	1.86						
Urea	6	49.6	2.29						
	9	45.3	2.15						

Least significant difference (0.05) for all treatments 8.7 barrels per acre.

Results from the field experiment given in table 6 show that a decrease in the yield of rice was obtained from turning under rice straw before planting. The lower yield probably occurred from the immobilization of some of the available nitrogen and the reducing conditions produced by the rice straw. The oxygen disappeared in a few days from the soil in all treatments after flooding. The E_H of the soil in the rice straw treatment decreased faster than the E_H of the other two treatments, figure 6. The growth of rice was noticeably poorer on straw-treated plots early in the season when the oxidation-reduction potential was decreasing rapidly. After the E_H leveled off at 50 millivolts and became equal to the values of the treatments not receiving rice straw, the growth became normal. The soil conditioner caused a decrease in yield.

In the nutrient solution experiment conducted in the laboratory, ammonium nitrogen was more readily absorbed by the rice than nitrate nitrogen regardless of whether oxygen or nitrogen was bubbled through the nutrient solution, figures 7 and 8. The nitrate form was absorbed faster from the solution saturated with nitrogen gas than from the oxygen-saturated solution. Ammonium nitrogen was more readily absorbed by rice plants from a solution saturated with oxygen than from a solution aerated with nitrogen gas. These data indicate that added oxygen in the root zone increased the absorption of ammonium nitrogen more than the absorption of nitrate nitrogen. This enhancement of

Figure 6.-The effect of a soil conditioner and rice straw on the E_H of a flooded Crowley silt loam.

Figure 7.-The ammonium nitrogen content of oxygen-saturated and nitrogen-saturated nutrient solutions containing rice.

Figure 8.-The nitrate nitrogen content of oxygen-saturated and nitrogen-saturated nutrient solutions containing rice.

ammonium nitrogen absorption was much more pronounced in this experiment than in the first greenhouse experiment. One reason for this may have been due to the oxygen having been supplied uniformly throughout the root zone in the nutrient solutions. In the greenhouse experiment it was present only in the flood water or in channels up through the soil.

The results from the laboratory experiment on the effects of nitrate and ammonium nitrogen on the intake of nitrogen by rice on Crowley silt loam are shown in table 7 and in figures 9 and 10. Both the added nitrate nitrogen and ammonium nitrogen disappeared from the soil and flood water with the ammonium nitrogen disappearing more rapidly, figure 9. The introduction of oxygen into the soil did not appear to materially affect the decrease in concentration of either of the nitrogen forms. The concentration of nitrates in the treatments receiving ammonium nitrogen and the concentration of ammonium nitrogen in the treatments receiving nitrates remained at a fairly constant low level throughout the experimental period.

The ammonium nitrogen was the form of nitrogen most readily absorbed by the rice plant. This can be observed in table 7 and figure 10 which show the per cent nitrogen in the rice from the different treatments and the amount of nitrogen absorbed by the rice from the different treatments. There did not appear to be any interaction between either of

Table 7.-Effect of source of nitrogen and oxidation treatment on the growth of rice, the nitrogen absorption by rice, and the nitrogen content of the soil in a laboratory experiment on Crowley silt loam.

		Source of nitrogen											
		NO ₃						NH ₃					
		Time in days						Time in days					
		0	3	5	7	9	14	0	3	5	7	9	14
Oxygen	Mgm. NO ₃ -N in soil	7.50	3.75	2.00	1.50	1.01	0.49	0.75	1.20	1.00	1.20	1.10	1.00
	Mgm. NH ₃ -N in soil	1.20	2.04	1.35	1.20	0.30	0.60	8.25	4.07	-	1.45	0.53	0.53
	Green wt. of plants (gms)	-	0.46	0.39	0.54	1.25	1.65	-	0.82	0.68	0.69	1.85	1.99
	Mgm. N absorbed	-	0.30	0.27	1.18	3.37	4.89	-	0.43	1.96	2.92	6.52	8.99
	% N (dry weight)	-	2.00	1.69	2.79	2.07	2.36	-	1.43	2.04	3.56	2.49	3.19
No oxygen	Mgm. NO ₃ -N in soil	7.50	5.00	3.75	2.25	2.25	1.20	0.75	1.20	1.20	1.00	1.00	1.05
	Mgm. NH ₃ -N in soil	1.20	1.80	2.03	0.86	0.68	0.52	8.25	1.58	1.55	1.14	1.05	1.00
	Green wt. of plants (gms)	-	0.43	0.40	0.83	0.74	1.94	-	0.39	0.68	1.10	1.08	2.28
	Mgm. N absorbed	-	0.16	0.44	1.94	1.98	4.42	-	0.00	2.35	3.83	5.37	8.88
	% N (dry weight)	-	1.82	1.80	2.67	2.13	1.99	-	1.57	2.52	2.66	2.36	2.91

Figure 9.-The effect of oxygen on the decrease of nitrate and ammonium nitrogen in a Crowley silt loam.

Figure 10.-The effect of the form of nitrogen and the effect of oxygen on the absorption of nitrogen by rice. Ordinate is milligrams of nitrogen absorbed per plant.

the forms of nitrogen and the oxygen introduced to the root zone of the rice plants. There was no interaction between the level of free oxygen and sources of nitrogen in their effects on the growth of the rice plants or the absorption of nitrogen by the plants. The lack of interaction was contrary to the results obtained from the nutrient solution experiment, in which oxygen increased the absorption of ammonium nitrogen.

The data in table 7 shows that slightly more nitrogen than was added to the soil in the ammonium treatments was absorbed by the rice plant during the 14-day observation period. Approximately two-thirds as much nitrogen was absorbed by the rice plants from the nitrate nitrogen treatments as was added in the form of sodium nitrate for the same 14-day period. Within the 14-day period which this test covered, ammonium nitrogen was utilized more efficiently by rice than was nitrate nitrogen. Both the nitrate and ammonium forms of nitrogen disappeared from the rice soil much more rapidly than nitrogen was being absorbed by the plant. Only near the end of the 14-day period did the amount of nitrogen absorbed plus the available nitrogen present in the soil approximate the amount added to the soil. This indicates that a lesser amount of nitrogen, extractable with normal sodium chloride solution, was present in the soil than was added in the treatments. The rice plant appeared capable of obtaining nitrogen from the soil even

though very little was present in the soil as the nitrate or ammonium form at any one time. This may be due to the rice plant's ability to utilize forms of nitrogen other than nitrate and ammonium, or it may be that these two forms are maintained at a low equilibrium concentration in the rice soil but are produced rapidly enough to compensate for their removal by the rice.

The rates of consumption of oxygen in suspensions of Crowley silt loam, as determined by the method previously described, are shown in figure 11. The oxygen consumption was determined on soil that had been treated in the following ways: (1) air-dried, (2) submerged for 30 days, and (3) submerged for 30 days with organic matter in the form of green clover added to the soil 10 days before the determinations were made. The pH of the air-dried soil was 6.2. The pH of the submerged soil was 7.0 while that of the submerged soil to which green clover was added was 7.1. There was a great difference in the rate of oxygen consumption among the three soil treatments. The air-dried soil consumed oxygen at the very slow rate of 1.18 parts per million per hour, whereas the soil flooded for 30 days consumed oxygen at the rate of 6.82 parts per million per hour, and the rate of consumption in the flooded soil to which organic matter had been added was 25.0 parts per million per hour. In all three cases, the rates of consumption of free oxygen were fairly constant, which indicate that the

Figure 11.-Oxygen consumption by suspensions of Crowley silt loam. The rates of consumption are the slopes of the curves.

Figure 12.-The relationship between the oxygen concentration and the E_H of suspensions of Crowley silt loam.

rate of reaction was not dependent on the oxygen concentration. The rate of the reaction was definitely dependent on the rate of oxidation of the reduced substances in the soil because the rate increased as the reduced products in the soil were increased by flooding and by the addition of readily decomposable organic matter. Since the oxygen concentration did not influence the reaction rate, the reaction order was zero with respect to oxygen. The order of the reaction with respect to the oxidation of the reduced products was greater than zero, but the reduced products were probably present in such an excess that their concentrations were not appreciably decreased through oxidation by the small amount of oxygen added.

The differences in the rates of consumption of oxygen from the differently treated soils was large enough for variations in the ability of the soils to reduce oxygen to be easily distinguished. It appears that this procedure may be used to advantage in measuring the intensity and perhaps the degree of reduction in a submerged soil.

In a rice field the oxygen moving down through the flood water to the soil surface is not being supplied as rapidly as the soil is capable of reducing this oxygen. Consequently, much of the oxygen being supplied to the soil surface is utilized for the oxidation of reduced products from microbial decomposition and is not available to the rice roots for active root respiration. It is quite possible

that increasing the movement of oxygen to the soil-water interface will increase the supply available to the rice plants without increasing the oxygen concentration to any appreciable depth, more than 1 centimeter, in the soil below the soil-water interface. This is the explanation for the observation that the treatments in the first greenhouse experiment which received oxygen produced a greater growth of rice than was obtained in the treatments not receiving oxygen, even though no oxygen was detected below the soil-water interface in either treatment.

There appeared to be a relationship between the free oxygen concentration and the E_H at any given time for the three soil treatments worked with, figure 12. When the E_H of the soil suspension was plotted as a function of the logarithm of the oxygen concentration, a linear relationship was obtained. This is in agreement with the theoretical relationship between the potential of a system and the concentration of the substances in the system as given by the Nernst equation:

$$E_H = E^0 + \frac{60}{n} \log \frac{(\text{Oxidized})}{(\text{Reduced})} \text{ at } 30^\circ \text{ C.},$$

where E^0 is the normal oxidation-reduction potential in millivolts and n is the number of electrons involved in the reaction.

The slopes of the E_H -log O_2 concentration equation were different for the three soil treatments. These slopes were 35, 75, and 205 millivolts per logarithmic unit for the soil

submerged 30 days, the soil submerged 30 days plus organic matter, and the air-dry soil, respectively. The reduction reaction by which oxygen is most likely reduced is

The amount of water formed should not influence the potential since it is present in excess in the system and thus for all practical purposes remains at a constant concentration. If 4 electrons are involved in the reduction of each molecule of oxygen, n in the Nernst equation would be 4. In this case, the slope of the E_H -log O_2 concentration would be 15 millivolts per logarithmic unit. The experimentally determined values listed above are greater than this figure. One explanation for this is that oxygen may not be completely reduced to water, in which case n would be a smaller figure than 4 and the theoretical slope of the above equation would be greater than 15 millivolts per logarithmic unit. Perhaps the most probable explanation is that the oxygen is reduced completely to water and the reaction is more involved than is postulated by the above equation.

SUMMARY AND CONCLUSIONS

A study has been made of the effects of the oxidation levels of the root substrate on the growth of rice, on the absorption of nitrate and ammonium nitrogen by rice, and on the stability of nitrate and ammonium nitrogen in soil.

Bubbling oxygen up through the flood water in rice cultures produced a significant increase in the growth of rice in a greenhouse experiment on Crowley silt loam. No increase in the free oxygen content of the soil was measured as a result of the addition of oxygen. The beneficial effects of the oxygen to the rice were restricted to the roots concentrated at the soil surface. Measurements made on a Crowley silt loam by a method developed during the course of the experiment showed that the rate of reduction of oxygen by the soil was increased considerably by submerging the soil and increased still further by adding organic matter to the submerged soil. There was probably competition between the rice roots and the reduced substances in the soil for the oxygen supplied by diffusion through the flood water from the atmosphere. Much of the oxygen that diffused to the soil-water interface therefore, was not available to the rice plant. This lack of availability of oxygen to the rice roots may have been due to the slow rate of diffusion of oxygen through the soil, the reducing conditions in the soil which reduced the oxygen as it reached the

soil, or both. Flooding the soil also caused a decrease in the oxidation-reduction potential, or E_H , of the soil. The rate of decrease of the E_H was highest where organic matter was added to the soil.

A method was developed for determining the rate of consumption of oxygen by a soil suspension. The free oxygen concentration of the soil suspension was closely related to the E_H of the suspension. A linear relationship was obtained when the E_H was plotted against the logarithm of the oxygen concentration.

Ammonium nitrogen was found to be more readily absorbed by rice than nitrate nitrogen both in the soil cultures and the nutrient solutions regardless of the oxidation level of the root medium. There did not appear to be any specific interaction between any of the oxidation treatments and the absorption of either nitrate or ammonium nitrogen by rice in any soil experiment. An increase in rate of absorption of ammonium nitrogen due to the addition of oxygen occurred in the nutrient solution experiment.

The application of organic matter to a Crowley silt loam enhanced the loss of ammonia by volatilization. This loss of ammonia was associated with a lowered E_H , an increased pH, and flood water temperatures as high as 100° F.

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Title of Thesis: Concentration and Movement of Oxygen as Related to Absorption
of Ammonium and Nitrate Nitrogen by Rice.

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