INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
RICE UNIVERSITY

THE USE OF REDOX MEASUREMENTS
TO STUDY METHANE MITIGATION OPTIONS
IN TEXAS RICE PADDIES

by

SANDRA LEWIS

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE
DOCTOR OF PHILOSOPHY

APPROVED, THESIS COMMITTEE:

Ronald L. Sass, Chairman, Professor
Ecology and Evolutionary Biology

Frank M. Fisher, Professor
Ecology and Evolutionary Biology

Philip R. Brooks, Professor
Chemistry

Houston, Texas
May, 1996
ABSTRACT

The Use of Redox Measurements to Study Methane Mitigation Options in Texas Rice Paddies

by

Sandra Lewis

Redox measurements were used to study whether different mitigation options affect methane emission and production by altering the electrochemical environment in rice paddy soil. These mitigation options include field drainage, use of different cultivars, and changing soil texture. Results indicate that the redox potential (Eh) is an accurate indicator of whether or not methane is produced. Also, the timing of methane production and emission was found to be dependent upon the reduction of iron and subsequent increase of the ferrous ion concentration. Field drainage is a mitigation option that successfully lowers methane emission rates by increasing the Eh. By studying the other mitigation options, it was found that once sufficiently negative Eh values are reached, different non-redox parameters control the actual amount of methane emitted.
ACKNOWLEDGMENTS

Special appreciation is extended to my advisors, Drs. Ron Sass and Frank Fisher, who have so patiently guided and encouraged me in my research. I also thank my third committee member, Dr. Phil Brooks, for his advice and helpful comments.

In addition, the other graduate students and researchers in my group deserve recognition. By working with Dr. George Byrd, Lief Sigren, Cylette Willis, and Huang Yao under the guidance of our advisors, I have had the opportunity to work in a truly inter-disciplinary science laboratory. The camaraderie and openness in the lab environment continually helped and challenged me.

Without the aid of undergraduate researchers and people at the Texas A&M University Agricultural Research and Extension Center, much of the data presented here would not exist. I would like to thank the following for their contributions: Patrick Conant, Patrick Eason, Yongping Gao, Mike Jund, Jennifer King, Michael Lightfoot, Alice Lim, Dan Martin, Oscar Ramirez, Alex Renwick, Adam Richardson, Don Robinson, Kara Robinson, Dr. Fred Turner, and Jeff Wu, and especially Amber Dunten, Melissa Henson, Steven Mora, Cam Smith, and Ammi Spencer.

Finally, my family and friends lent the moral support I really needed over the last five years. I would like to thank all who gave me their time, care, and prayers, especially my husband, Larry. Most of all, I am thankful to my Lord and Savior, Jesus Christ, for leading me here five years ago and enabling me to finish. It is to Him that all glory goes.
TABLE OF CONTENTS

Acknowledgments........................................................................................................iii
List of Figures..................................................................................................................vi
List of Tables..................................................................................................................ix

Chapter
1. Introduction....................................................................................................................1

2. Field Site Description and Methods..............................................................................14
   2.1. Description of Field Sites.......................................................................................14
       2.1.1. 1993 Field Layout and Season Description.................................................15
       2.1.2. 1994 Field Layout and Season Description.................................................17
       2.1.3. 1995 Field Layout and Season Description.................................................19
   2.2. Soil Redox and pH Measurements...........................................................................21
       2.2.1. Ferrous Iron Concentration Determinations .................................................21
       2.2.2. In-Situ Redox Measurements.......................................................................23
       2.2.3. Soil pH Measurement..................................................................................27
   2.3. Laboratory Potential Methane Production Estimations from
       Field Collected Soil Samples....................................................................................27
   2.4. Total Organic Carbon Determinations ....................................................................29
   2.5. Soil Metals Analysis..............................................................................................32
   2.6. Most Probable Number Analysis..........................................................................33
   2.7. Root Distribution..................................................................................................36
   2.8. Agal Mat Development.........................................................................................37
   2.9. Outdoor Pot Experiment.......................................................................................38
3. Results of Seasonal Trends
   3.1. Eh Results
   3.2. Ferrous Ion Concentration Results
   3.3. Results of pH
   3.4. Methane Production Results

4. Results of the Effect of Field Drainage
   4.1. Overall Results on the Effects of Drainage
   4.2. Variation by Depth During the Drain

5. Results of Cultivar Differences
   5.1. Differences Between Rice and Non-Rice Plots
   5.2. Cultivar Differences

6. Results of the Impact of Soil Texture
   6.1. Soil Characteristics
   6.2. Redox Characteristics
   6.3. Methane Production Rates
   6.4. Algal Mat, Root Distribution, and MPN Analysis

7. Discussion
   7.1. Seasonal Patterns
   7.2. Field Drainage Effects
   7.3. Cultivar Differences
   7.4. Soil Texture Effects
   7.5. Conclusions

Bibliography
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1. Iron Eh-pH Diagram</td>
<td>11</td>
</tr>
<tr>
<td>2.1. 1993 Field Site Diagram</td>
<td>16</td>
</tr>
<tr>
<td>2.2. 1994 Field Site Diagram</td>
<td>18</td>
</tr>
<tr>
<td>2.3. 1995 Field Site Diagram</td>
<td>20</td>
</tr>
<tr>
<td>2.4. Ferrous Iron Measurement Calibration Curve</td>
<td>24</td>
</tr>
<tr>
<td>2.5. The Effect of Temperature on Eh of Standardizing Solution</td>
<td>26</td>
</tr>
<tr>
<td>2.6. Diagram of Total Organic Carbon Apparatus</td>
<td>30</td>
</tr>
<tr>
<td>3.1. Average Daily Eh vs. Time</td>
<td>42</td>
</tr>
<tr>
<td>3.2. Eh by Depth vs. Time</td>
<td>44</td>
</tr>
<tr>
<td>3.3. Average Daily Ferrous Ion Concentration vs. Time</td>
<td>46</td>
</tr>
<tr>
<td>3.4. Ferrous Ion Concentration by Depth vs. Time</td>
<td>47</td>
</tr>
<tr>
<td>3.5. Average Daily pH vs. Time</td>
<td>49</td>
</tr>
<tr>
<td>3.6. Soil pH by Depth vs. Time</td>
<td>50</td>
</tr>
<tr>
<td>3.7. Methane Production Rates by Depth vs. Time</td>
<td>52</td>
</tr>
<tr>
<td>4.1. Methane Production Rates, Eh, Ferrous Ion Concentration, and pH vs. Time for the 1994 Season</td>
<td>56</td>
</tr>
<tr>
<td>4.2. Methane Emission Rates and Eh vs. Time for the 1994 Outdoor Potted Rice Study</td>
<td>58</td>
</tr>
<tr>
<td>4.3. Eh and pH vs. Time for the 1995 Rosenberg Season</td>
<td>60</td>
</tr>
<tr>
<td>4.4. Eh by Depth vs. Time During a Drain Event (China, Texas)</td>
<td>63</td>
</tr>
<tr>
<td>4.5. Ferrous Ion Concentration by Depth vs. Time During a Drain Event (China, Texas)</td>
<td>65</td>
</tr>
<tr>
<td>4.6. Methane Production Rates by Depth vs. Time During a Drain Event (China, Texas)</td>
<td>67</td>
</tr>
</tbody>
</table>
4.7. Eh by Depth vs. Time During a Drain Event (Rosenberg).............68
4.8. pH by Depth vs. Time During a Drain Event (Rosenberg)...........70
5.1. Eh vs. Time for Rice and Non-Rice Plots..................................73
5.2. Ferrous Ion Concentration, pH and %TOC vs. Time for Rice
    and Non-Rice Plots.....................................................................75
5.3. Average Eh vs. Time for Ten Cultivars in 1993.........................77
5.4. Average Eh vs. Time for Two Cultivars in 1995..........................79
5.5. Average Ferrous Ion Concentration vs. Time for Two Cultivars
    in 1995....................................................................................81
5.6. Average pH vs. Time for Two Cultivar Groups in 1993...............82
5.7. Average pH after 30 DAF vs. Cultivar Position..........................84
5.8. Average Seasonal %TOC vs. Cultivar Position...........................86
5.9. Methane Production Rates vs. Time for Two Cultivars in 1995.....88
6.1. Soil Texture for 1994-95 China, Texas Fields.............................90
6.2. Eh, Ferrous Ion Concentration, and pH vs. Time for the North
    Center and South Fields in 1994..............................................93
6.3. Eh vs. Time for the North, Center and South Fields in 1995.......95
6.4. Methane Production Rates vs. Time for the North, Center,
    and South Fields in 1994-95..................................................96
6.5. Mean Root Weights vs. Time for the North and South Fields
    in 1994.............................................................................98
6.6. MPN Results for the North, Center and South Fields in 1994-95...99
7.1. Methane Emission Rates and Eh vs. Time for.........................103
7.2. The Standard Deviation of the Eh and Methane Emission Rates
    vs. Time.............................................................................104
7.3. Eh, Ferrous Ion Concentration, Methane Production and Emission
    Rates vs. Time...................................................................108
7.4. Ferrous Ion Concentration and pH by Depth vs. Time in 1994........110
7.5. Ferrous Ion Concentration and pH by Depth vs. Time in 1993........112
7.6. Average Redox (pH+pH) vs. Time..............................................120
7.7. Average Methane Emission Rates vs. Time for Ten Cultivars.......130
LIST OF TABLES

Table                                                   Page
1.1. List of Redox Systems Occurring in Paddy Soils..........................2
3.1. Comparison of Methane Production Rates Between 1994-95..........53
4.1. Record of Seasons Studying Field Drainage and Measured
     Parameters..............................................................................54
4.2. Significance Levels for the Eh, Ferrous Ion Concentration, and
     Methane Production During Field Drainage in 1994.............62
4.3. Significance Levels for the Eh and Ferrous Ion Concentration
     Post-Hoc Test Results During Field Drainage in 1994........64
5.1. Average %TOC Values for Non-Rice, Rice, and Lemont Plots......76
5.2. Pairwise Comparison Probabilities for Cultivar Soil pH
     Differences...............................................................................83
5.3. Pairwise Comparison Probabilities for Cultivar Soil %TOC
     Differences...............................................................................85
6.1. Concentrations of Soil Metals.....................................................92
7.1. Date of Initial Methane Emissions and Corresponding Eh Values
     Correlations............................................................................101
7.2. Average Methane Emission Rates in Rice and Non-Rice Plots.....125
1. INTRODUCTION

Rice is the world’s most important cultivated wetland crop, with the world annual production in 1990 of 518 million tons (Neue, 1993). More than 90% of the world’s rice production is in Asia. In East Asia, almost all rice is grown on irrigated land, and in Southeast and South Asia, 30-40% of the rice is grown on irrigated land (Neue, 1993). Irrigated lands are often used since the grain yield is much higher in submerged fields than in upland dry fields (Yoshida, 1981). This may arise from several factors. First, weed growth is reduced in flooded fields. Also, flooding produces an environment for the rice plant that is rich in soluble nutrients normally kept from the rice plant in dry fields.

The increased availability of nutrients occurs as the water forms a gas transport barrier between the soil and the atmosphere. Oxygen, the electron acceptor for aerobic respiration, is depleted within hours by microorganisms in the soil (Yoshida, 1981; Ponnampemerima, 1972). Once oxygen disappears, a series of inorganic compounds are consumed by different microbial organisms. Table 1.1 shows these redox couples and the relative state of reduction at which they occur. Each redox couple is fully reduced before the next electron acceptor is utilized.

The transition from aerobic to anaerobic conditions promotes growth of the rice plants in that several of the reactions produce soluble nutrients that are not normally available in aerobic soils. Concentrations of phosphorus, potassium, iron, manganese, and silicon usually increase after flooding which are needed by the rice plant (Yoshida, 1981).
Table 1.1. List of redox systems occurring in paddy soils. The list proceeds from those occurring in oxidized soils to those occurring only in highly reduced soils. (Yu, 1985; Patrick, 1981).

<table>
<thead>
<tr>
<th>Redox system</th>
<th>State of Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1/4O_2 + H^+ + e^- = 1/2H_2O$</td>
<td>Oxidized</td>
</tr>
<tr>
<td>$1/2NO_3^- + H^+ + e^- = 1/2NO_2^- + 1/2H_2O$</td>
<td>Moderately Reduced</td>
</tr>
<tr>
<td>$1/2MnO_2 + 2 H^+ + e^- = 1/2 Mn^{2+} + H_2O$</td>
<td>Reduced</td>
</tr>
<tr>
<td>$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$</td>
<td></td>
</tr>
<tr>
<td>$1/8 SO_4^{2-} + 5/4H^+ + e^- = 1/8H_2S + 1/2H_2O$</td>
<td>Reduced</td>
</tr>
<tr>
<td>$1/8CO_2 + H^+ + e^- = 1/8CH_4 + 1/4H_2O$</td>
<td></td>
</tr>
<tr>
<td>$H^+ + e^- = 1/2 H_2$</td>
<td>Highly reduced</td>
</tr>
</tbody>
</table>

There are negative consequences to the reduction, however, especially when highly reducing conditions are reached. The large amount of soluble ferrous iron sometimes leads to iron toxicity to the plant (Yoshida, 1981). In addition, the health of the rice roots is threatened in highly reduced soils, as oxygen, required for their respiration and growth, is not normally present. Fortunately, the rice plant has several adaptations that can reduce these negative consequences of flooding. These include the ability to transport oxygen from the atmosphere to the reduced root zone and the growth of special respiring roots near to the soil-water interface where oxygen is more available (Yoshida, 1981).

Another consequence of flooding is the formation of methane (CH4). The production and release of methane from rice fields may be responsible for 10-20% of all global methane emissions (Duxbury et al., 1993; IPCC, 1992; Wang et al., 1990; Cicerone and Shetter, 1981). The concentration of atmospheric methane has more than doubled in the past 200 years (Raynaud et al., 1988; Khalil and Rasmussen, 1987; Pearman et al., 1986). This increase is creating concern for global climate change since methane is
a known greenhouse gas. It has also been found to affect tropospheric and stratospheric ozone (Khalil, 1993a; Wuebbles and Edmonds, 1991).

Methane is produced by obligate anaerobic bacteria, which cannot survive in the presence of oxygen, once highly reduced conditions in the soil are reached. These bacteria are generally categorized as methanogenic bacteria. Methanogens can be divided into the following groups depending on their carbon source: those reducing carbon dioxide with hydrogen as an electron donor and those reducing methylated compounds, such as acetate (Boone et al., 1993). Acetate is used as both the electron donor and acceptor in what is known as the aceticlastic pathway (Boone et al., 1993). These reactions follow with the energy released by each reaction:

1. \[ \text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad (\Delta G^o = -131 \text{ kJ/mol}) \]  
   (Thauer et al., 1993)

2. \[ \text{CH}_3\text{COO}^- + \text{H}^+ \rightarrow \text{CH}_4 + 3\text{CO}_2 \quad (\Delta G^o = -36 \text{ kJ/mol}) \]  
   (Keltjens and Vogels, 1993)

Using concentrations typical of anaerobic ecosystems and not standard states, the free energies of these reactions are -3.2 and -24.7 kJ/mol, respectively, and the aceticlastic pathway becomes the energetically favored pathway (Brock and Madigan, 1991). This is due to the low hydrogen concentration present in anaerobic soils, generally observed to be lower than 10^{-3} atm (Brock and Madigan, 1991). Hydrogen is a very short-lived species in the soil, as the fermentative reactions which form it are spontaneous only when it is immediately consumed (Brock and Madigan, 1991). Isotope studies by Takai (1970) have described the aceticlastic pathway as the dominant mechanism, responsible for 75-90% of methane production in rice fields. However, more recent studies by Schütz
et al. (1989) reveal that carbon dioxide reduction may have a larger contribution, between 30-50%. The competition between these two types of methanogens may vary from field to field.

In the soil, acetate ions are produced through the decomposition of organic material, such as glucose. The main pathway of glucose degradation is the Embden-Meyerhoff (glycolytic) pathway followed by methanogenic acetate cleavage (Krumböck and Conrad, 1991). Cellulose and other polysaccharides may be hydrolyzed to sugar monomers, thereby providing an additional source of substrates. Simple sugars or amino acids, as well as larger polysaccharides and proteins may be derived from decaying algal, plant, or microbial biomass, originating either in the soil environment or floodwater (Personal communication, Dr. Frank Fisher). This source may be especially important late in the season when senescence is occurring more frequently.

Another source of organics includes materials released from the roots, as what are generally classified as root exudates. These include a wide variety of organic compounds released from the roots, including leakages, secretions, mucilages, mucigel and lysates (Hale et al., 1971). Roots have been found to naturally release organics, however, the amount may increase in conditions of high water stress and high microbial activity, as found in rice fields (Vancura, 1964; Grineva, 1962). Compounds leaked from roots normally include carbohydrates, organic acids and amino acids (Vancura and Hovadik, 1965).

Once methane is formed in the soil, it is eventually either moved to the atmosphere or oxidized to carbon dioxide by methane oxidizing, or methanotrophic bacteria. The air channels, or aerenchyma, that support the flow of oxygenated air from the atmosphere to the roots also allow the
diffusion of methane from the soil to the atmosphere. Other transport mechanisms include its diffusion through the water or through bubbling. Schütz et al. (1989) estimated that 96% of the methane is transported through the rice plant.

Estimates for the percentage of methane that is oxidized to CO2 range from 10-90% (Holzapfel-Pschorn et al., 1985; Sass et al., 1990; Schütz et al., 1989). This range is large due to many factors, including when during the season the samples were taken. These oxidation estimates are primarily derived from the difference between field measured methane emission rates and laboratory measured methane production rates. In-situ production rates are very difficult to measure. Therefore these oxidation percentages are rough estimates.

The amount of methane emitted from rice fields is expected to increase in the future as a 65% increase in rice demand is expected by 2020 (IRRI, 1988). This increased demand will need to be met by intensifying current cultivation, and therefore will probably result in higher levels of methane emissions. Several options for the mitigation of emissions from rice fields are currently under study. These include the use of different rice cultivars, varying sand/clay/silt percentages (or soil texture), and field drainage regimens. Before these mitigation options are fully implemented, it is advantageous to understand the mechanisms by which they decrease methane emissions. The National Inventories of CH4 and N2O (Khalil, 1993b) stated that four principles must be adhered to when investigating mitigation options:

1. Yield should not be decreased, and probably increased, by a mitigation practice.
2. There should be some additional benefit to the farmer, such as better water utilization or reduction of labor.
3. The rice varieties used should be desired by local consumers.
4. The mitigation practice should not increase emissions of other greenhouse gases, particularly nitrous oxide (N2O).

The use of different rice cultivars may be an important mitigation option, as cultivars have been found to emit different levels of methane (Unpublished data, Dr. Ron Sass). Methane emission rates may differ among cultivars due to varying amounts methane produced in the soil. Varieties with higher root exudation rates may stimulate greater methane production rates.

In addition, oxidation rates may differ among cultivars. The aerenchyma system of rice plants vary considerably, with some cultivars transporting larger amounts of oxygen to the soil. High transport plants may allow more oxygen to reach the soil thereby facilitating larger amounts of methane oxidation. Also, the sulfide oxidizing bacteria Beggiatoa, which are catalase negative, are known to reside in the rhizosphere of rice plants and may affect the amount of oxygen present in the root zone (Brock and Madigan, 1991). Their growth may be stimulated by the release of catalase from the rice roots. Different cultivars may have varying Beggiatoa populations associated with their root zones contributing to the oxygen concentration.

Besides transporting higher amounts of oxygen to the root zone, larger aerenchyma systems may also allow greater amounts of methane to be transported to the atmosphere. The fluxes of gas through the plant also depend upon the concentration gradients and diffusion coefficients of the roots and other areas inside the plant, the number of tillers per area, as
well as root biomass (Neue, 1993). All of these factors may vary by cultivar.

Changes in the soil texture has also been found to alter the methane emission rate. Though this may not be a practical mitigation option, there are many benefits to studying the mechanism behind a parameter which produces varying amounts of methane. Soil texture defines the sand/clay/silt ratio of soil. Clay particles are those which have a diameter smaller than 0.002 mm. Silt particles are 0.02-0.002 mm. Sand particles are larger than 0.02 mm, ranging up to 2 mm (Baize, 1993). The size of particles in the soil may alter the rates of diffusion of water through the soil as well as the growth of roots, and thereby impact the extent of reduction.

Methane emission rates have been found to vary significantly among fields of different soil texture. Sandier soils have been found to emit greater amounts of methane (Sass et al., 1994; Parashar et al., 1991; Yagi and Minami, 1990). Using data from four years and three different soil types, seasonal methane emissions ranged from 15.1 to 36.3 g CH4 m-1 in soil with sand content ranging from 18.8% to 32.5% (Sass et al., 1994).

It is presently unknown why the methane emissions vary with soil texture. It may be due to higher entrapment of methane occurring in clayey soils, thereby increasing oxidation (Sass et al., 1994). Another explanation may be that sandy soils produce more methane due to greater diffusion of substrates.

Finally, field drainage is considered a very viable option for mitigating methane emissions from rice fields. Many studies have shown that field drainage, by allowing air to re-enter the soil, dramatically reduces methane emissions (Sass et al., 1992; Yagi et al., 1992). Sass et al.
(1992) found a 50% decrease in methane with one mid-season drainage. However, it is not yet known whether the reduction in methane emissions is due to a reduction in methane production or an increase in methane oxidation.

The usefulness of field drainage is hindered by two negative consequences. Field drainage has been found to lower grain yield when the drainage occurs during the rice plant's reproductive stage (Yoshida, 1981). Field drainage also causes the soil redox state to be suitable for the production and emission of nitrous oxide (N2O) which is another greenhouse gas. These negative aspects must be addressed in any drainage study.

The mechanisms behind these mitigation options require the use of experiments that can monitor and detail electrochemical changes in the rice paddy soil environment. Several redox parameters to be used in these studies include the oxidation-reduction potential (or Eh), the ferrous ion concentration, and the pH. Other tools used are the measurement of the methane production rate in the soil, the total organic carbon fraction in the soil (TOC), the most probable number (MPN) bacteria population count, and soil texture analyses.

The redox parameters mentioned above have been widely used in the study of submerged soils (Ponnamperuma, 1972; Patrick, 1981). The Eh is a measure of the reduction intensity, or the ease of reduction. It is measured with a meter connecting platinum and reference electrodes, which are both inserted into the soil. It is often an important tool used in rice fields to determine plant growth and grain yield. Eh values of +700 to +400 mV are considered to be representative of oxidized systems and are average for aerated soils. Moderately reduced oxidized soils have an Eh in
the range of +400 to +100 mV. Reduced soils are typically in the range of +100 to -100 mV, and highly reduced soils are in the range of -300 to -100 mV (Patrick, 1981).

The Eh is very useful in indicating the dominant redox couple in the soil. Upon flooding, the Eh drops from values common in aerobic soils, and reaches values as low as -250 mV within two weeks after flooding (Ponnamperuma, 1981). This decrease in Eh is affected by the presence of organic matter, the concentrations of inorganic compounds that serve as alternative electron acceptors, and the soil temperature (Ponnamperuma, 1981).

Laboratory experiments suggest that iron reduction begins near +100 mV (Patrick and Henderson, 1981). Methane production has been found to begin closer to -150 mV (Wang et al., 1993), although some studies have shown methane production can be initiated at values closer to -100 mV (Mayer and Conrad, 1990).

The measurement of the ferrous ion content in the soil is another method of quantifying the state of reduction in the soil, known as the reduction capacity or the amount of reduction occurring in the soil. The reduction capacity is typically quantified by the partial pressure of oxygen in the soil. In flooded soils where oxygen is absent or present in very low concentrations, the reduction capacity can be measured by the concentration of reduced iron. The iron equilibrium is dominant in most paddy soils due to the large amount of bioreducible iron present (Patrick and Reddy, 1978).

Ferrous ion concentration trends upon flooding of rice fields have been well documented (Ponnamperuma, 1981; Patrick, 1981). Ponnamperuma (1981) measured the trends of the water-soluble ferrous
ion fraction, which is the most active form, and detected an exponential increase during the first three weeks after submerging followed by a leveling off or decrease. The ferrous ion concentration either levels off or decreases depending upon the temperature and physical properties of the soil (pH, percent iron, organic matter, and the salt concentration in the soil) (Ponnampenura, 1985).

Figure 1.1 is an Eh-pH diagram which displays common ferric complexes, as well as the Eh and pH values at which they are stable. Equation 1.1 shows the main reaction responsible for the increase in ferrous ion concentration in paddy soils:

\[
\text{Fe(OH)}_3 + 3\text{H}^+ + e^- = \text{Fe}^{2+} + 3\text{H}_2\text{O} \quad (1.1)
\]

Amorphous Fe(OH)\(_3\) is the main source of ferric ion for the initial increase after flooding in acid soils, since it is the stable ferric component of soils with pH values below 7. This increase is accompanied by an increase in pH as three hydrogen ions are consumed in the reaction.

Ferric oxide complexes become unstable at an Eh near 100 mV (Patrick and Henderson, 1980). The presence of ferric complexes causes the Eh to remain between 100 and -100 mV, and therefore buffers the redox potential from further decrease (Patrick, 1981). This buffering ensures a sustainable environment for the growth of the rice plants. Toxins harmful to the roots of rice plants, such as sulfides and organic acids, are produced at redox potentials below -150 mV, as obligate anaerobic and fermentative bacteria become dominant (Patrick, 1981). Microorganisms that utilize nitrates, manganese (IV) complexes, and ferric complexes as electron acceptors usually produce only CO\(_2\) and reduced ions, and
Figure 1.1. The Eh-pH diagram for the iron equilibrium in submerged soils, relative to an aqueous Fe$^{2+}$ activity of one millimole at 25°C (Ponnampерuma, 1967).
therefore they present no harm to the rice plants (Patrick, 1981). Hence, the buffering effect of the ferric-ferrous equilibrium has proved valuable in its ability to serve as both an accurate indicator of the redox capacity of the soil and a force in maintaining conditions suitable for rice growth.

The pH is another useful indicator of the electrochemical changes in soil after flooding. Upon flooding, the pH has been found to increase in acid soils and decrease in alkaline soils, normally resulting in near-neutral conditions (Ponnampetruma, 1972; Ponnampetruma, 1981). The increase of pH in acid soils is due to the iron reduction processes described above. Reaction 1.1, which is responsible for the initial increase of ferrous ions, shows three hydrogen ions being consumed in the reductive process. The drop in pH of alkaline soils is predominantly caused by the build up of CO2 and the dominance of the carbonate equilibria described below.

The optimum pH for methane production was observed to be between 6.5 and 7.5 in laboratory conditions (Wang et al., 1993). Ponnampetruma (1981) found that the pH of most paddy soils is between 6.7 and 7.2 at a few weeks after flooding and is maintained at these levels primarily by the carbonate equilibria. In cases where low levels of oxygen persist, the accumulation of CO2 is usually encountered (Neue, 1991; Bohn et. al., 1985). Ponnampetruma (1981) observed that between one to three metric tons of CO2 are produced in the plowed layer of paddy soil in a one hectare area during the first three weeks following flooding. The final CO2 concentration is dependent upon the temperature of the soil and the amount of organic matter present, as well as the percentage of iron and manganese.
Since CO$_2$ is very soluble in water, it readily equilibrates to carbonic acid, H$_2$CO$_3$. This then equilibrates with bicarbonate ions, HCO$_3^-$, and maintains the near neutral pH by the following equilibrium:

$$\text{H}_2\text{CO}_3 = \text{H}^+ + \text{HCO}_3^- \quad \text{pka}=6.4 \quad (1.4)$$

In this equation, H$_2$CO$_3$ represents all three forms of CO$_2$ found in solution: H$_2$CO$_3$, CO$_2$, and CO$_2$(aq).

Organic matter can also contribute to the maintaining or lowering of the pH. It is through the decomposition of organic residues that reduction and the initial increase of pH occurs. However, later in the season, it is probable that organic acids accumulate as products of fermentative decomposition pathways. Products of glucose metabolism include propionate, ethanol/butyrate, succinate, and lactate (Krumböck and Conrad, 1991). These then cause a decrease in pH, especially in soils rich in organic material and at times during the growing season when decomposition rates are high (Cang et al., 1985).

Redox parameters, such as the Eh, ferrous ion concentration, and pH, are valuable indicators of the reduced state of paddy soil. Evidence indicates that certain mitigation options are effective because they change the redox state of the soil, decreasing the probability of methane production. This study is concerned with using redox parameters as tools for uncovering the mechanisms behind the mitigation options.
2. FIELD SITE DESCRIPTION AND METHODS

Experiments were conducted over three field seasons, 1993-1995, and in an outdoor potted rice study in 1994. The field descriptions and methods for the collection and analyses of samples are described in the following subsections. Methane flux determinations were determined from gas samples taken in the field and pots. Methane production measurements and other soil characteristics were measured in the laboratory from soil cores taken in the fields and pots. In-situ monitoring of the Eh is also described.

2.1. Description of Field Sites

The majority of the fields used in this study are located in the Texas A&M University Agricultural Research and Extension Center near Beaumont, Texas, which is at longitude 94°30′W, latitude 29°57′N. Rice is the main crop for this area, as many factors make the area suitable for its growth. The annual growing season of the area is 275 days, with only 15 days annually that have temperatures below 0° C. Annual rainfall is high, averaging 122 mm per month during the rice-growing season (Sass et al., 1990). Also, the land is level, has poor internal drainage, and other physical-chemical properties that make it similar to soils in other rice-growing areas around the world (Sass et al., 1990).

Water in the fields was maintained under management practices similar to those used for commercial rice fields of the southern United States. This included brief flush irrigations to maintain adequate soil
moisture until the plants reach 15 cm in height (30-40 days after seedling emergence) and thereafter a 10-cm flood was maintained.

In 1995, another field was also studied in Rosenberg, Texas. Rosenberg is approximately 30 miles southwest of Houston. Rice is a major crop for the area. The soil was found to have an average sand:clay:silt ratio of 39:23:38.

2.1.1. 1993 Field Layout and Season Description

In 1993, ten different rice cultivar plots and one non-rice plot were monitored. The cultivars include Lebonnet, Lemont, Dawn, Katy, Della, IR 36, Mars, Brazos, Labelle, and Jasmine 85. Figure 2.1 shows a diagram of the 1993 field lay-out. Each field was approximately 5 meters wide and 30 meters long. Planting occurred on April 27, 1993. Seeds were drill-planted at 112 kg ha\(^{-1}\) in rows spaced 20 cm apart. They were planted in predominantly clayey soil designated as Lake Charles, a thermic Typic Pelludert (Vertisol), which has an average sand-clay-silt ratio of 22:35:43 (Sass et al., 1994).

The Jasmine field had been cultivated with Jasmine rice for two previous years (1991-92). The remaining soils had been fallow since 1989 (Sass et al., 1994). Flooding occurred on June 7, 1993. Harvesting of the cultivars occurred between August 17 and September 2 (71-87 days after flooding, or DAF), and final field drainage was on September 2 (87 DAF).
Figure 2.1. Diagram of rice field plots for experiments in the 1993 season. Numbers are distances in meters. E1i sampling was performed near the boardwalks labeled with an "x". Soil cores were taken randomly within a 5 meter distance from the boardwalk labelled "S". Numbers within the fields designate the cultivar position in the south-north direction.
2.1.2. 1994 Season Description and Field Layout

In 1994, three of the ten cultivars used in 1993 were planted - Lemont, Labelle, and Mars. These were cultivated in adjacent plots in three separate fields designated as north, center, and south (see Figure 2.2). My work was in the three Lemont fields, with some sampling in Mars and Labelle Center late in the season. Each field was approximately 4 meters wide and 57 meters long.

These cultivars were planted on April 5, 1994, in the same manner as the 1993 crop. Seeds were drill-planted at 118 kg ha\(^{-1}\) in approximately 15 rows spaced 20 cm apart. The seedling density was 192±34.0 (SD) tillers m\(^{-2}\) for the three fields (Personal communication, Huang Yao). Nitrogen as urea was applied three times: 50 lbs N ha\(^{-1}\) on April 5 (just after planting), 70 lbs N ha\(^{-1}\) on May 10 (7 days before permanent flood), and 45 lbs N ha\(^{-1}\) on June 10 (10 days before panicle differentiation).

These fields were along a soil gradient from a sandy end (north) to a more clayey end (south). The sandy north end was primarily soil designated as Bernard-Morey, which is a thermic Vertic Ochraqualf (Mollisol) and has an average sand-clay-silt ratio of 29:25:47 (Sass et al., 1994). The Bernard-Morey soil merged with more clayey soil at the south end. At this location, there may have been a mixture of Bernard-Morey, Lake Charles, and Beaumont soils. Beaumont soil is a thermic Entic Pelludert (Vertisol) and has an average sand-clay-silt ratio of 4:65:31 (Sass et al., 1994).

The north field had been fallow for one year prior to this season, but it had been planted for two years prior to that (1991-92). The center field had been also fallow the previous season, however, it had only been used
Figure 2.2. Diagram of rice field plots for experiments in the 1994 season. Redox studies were only performed in the three Lemont fields this season. Numbers are distances in meters. Eh sampling was performed near the boardwalks labelled with an "x". Soil cores were taken randomly throughout the length of the entire field.
one year before that (1992). The south field had been fallow for many years before use in 1994.

Flooding occurred on May 17, 1994. The north and south fields were drained twice during the season, while the center field was kept continually flooded with its water level maintained at 10 cm. The first field drainage occurred from June 16-June 21 (30-35 DAF), and the second occurred from July 20-July 25 (64-69 DAF). Lemont and Mars were harvested on August 11 (86 DAF), with the final drainage on August 17 (91 DAF).

2.1.3. 1995 Season Description and Field Layout

In 1995, four cultivars were planted in the same north, center, and south fields used in 1994. The four cultivars were Lemont, Della, Mars, and Cypress, in order over an east to west gradient (see Figure 2.3). I monitored redox potential and methane production in the three Lemont fields and the three Mars fields, as well as in a non-rice plot (bare center). The rice seeds were drill planted in 10 rows 20 cm apart on April 19, 1995. Flooding occurred on May 30, and harvesting occurred on August 16-17 (78-79 DAF), with final field drainage occurring on August 21 (83 DAF). A continual flood was maintained throughout the season.

During this season, another field site was monitored in Rosenberg, Texas. Two plots were cultivated using the cultivar Lemont. Rice was planted in rows 18 cm apart with every third row of rice omitted. One was continually flooded, and one experienced a mid-season drain. These fields were planted on April 24, 1995. Flooding occurred on May 25, and draining prior to harvest occurred on August 11, or 78 DAF. Mid-season draining occurred from July 14-July 20, or 50-56 DAF.
Figure 2.3. Diagram of rice field plots for experiments in the 1995 season. Redox studies were performed in the three Lemont fields, the three Mars fields, and in the non-vegetated center plot this season. Numbers are distances in meters. Eh sampling was performed near the boardwalks labelled with an "x". Soil cores were taken randomly throughout the length of the entire field.
2.2. Soil Redox and pH Measurements

2.2.1. Ferrous Iron Concentration Determinations

Three cores were taken from each field sampled, one quarter of the way from a rice row. Samples were collected using coring devices made from 60-cm³ plastic syringes from which the needle-end had been removed and the wall sharpened as first described by Sass et al. (1990). The corers were pushed into the soil until filled, then they were stoppered and pulled out. The cores taken were ten cm in length and 2.8 cm in diameter. The cores were transferred to intact 60 cc syringes which were sealed with a plunger to maintain anaerobic field conditions. The cores were immersed in a bucket of field water and transported back to the laboratory within four hours where they were frozen until analysis.

After thawing, the cores were divided into the desired segments, 5 cm in 1993 and 2.5 cm segments in 1994. The soil from the specific depths were then homogenized with a spatula before analysis. In 1993, the three cores were homogenized and analyzed separately. In 1994, the different depths from all three cores were mixed together and then analyzed.

Statistical analysis of the data from both years (1993-94) indicate that the variance associated with homogenization within the syringe is not comparable to the variance from homogenization among different syringes (p<0.01)\(^1\). The average normalized standard deviations for 1993 and 1994 were 10.9 (±7.9, SD) and 8.3 (±8.1, SD), respectively. This suggests that the amount of spatial heterogeneity occurring within one syringe is not the

\(^1\) An unpaired t-test was used to compare the percent error [(standard deviation/mean)\(*100\)] for 1993 and 1994 data, the numbers of samples were 140 and 195, respectively.
same as among syringes taken at different locations within the field. The heterogeneity within one syringe may not be representative of the heterogeneity of the field, and that the larger average percent error in 1993 is due to the heterogeneity of the field.

The change in experimental procedure in 1994 was effected to minimize the variance from field heterogeneity. The error was lowered by the homogenization of individual syringes during the 1993 season. However, the best estimation of error from experimental handling comes from the 1994 season.

Concerns for whether the shift in methods would cause a difference in the mean as well as in the error prompted experiments to test the similarity of both methods. In 1994, groups of cores were analyzed using both methods. A two-way ANOVA, with depth and method as parameters, resulted in no significant differences between the two methods.

Ferrozine (3-(2-Pyridal)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine) has been found to be an accurate indicator of ferrous ion concentrations in aquatic sediments (Stookey, 1970). This method was taken from Lovely and Phillips (1987). In this procedure, a known amount of soil (approximately 0.1 g) was added to a 10 mL glass vial. Five ml was added of either 0.5 M HCl. Three replicates were taken of each soil sample.

The bottles were capped and shaken to disperse the soil uniformly throughout the liquid. If necessary, a spatula was used to break up any soil chunks. They were allowed to sit in the laboratory for one-two hours. Then, five ml of a ferrozine-buffer solution was added to a cuvette. The solution was composed of 1 g/L of ferrozine and 11.9155 g/L of Hepes (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]). Hepes is a commonly used biological buffer with a pKa of 7.5 (at 25 °C) and is known
to have a useful pH range of 6.8-8.2. To the cuvette, 0.1 mL of the soil-HCl mixture was added. All the cuvettes were then shaken on a mini-shaker and filtered through Whatman number 2 filter paper into a clean cuvette. This was next measured in a spectrophotometer (Bausch and Lomb Spec 120) at a wavelength of 562 nm. A calibration curve was made measuring a set of ferrous ion solutions (2-90 mg/L FeSO4·7H2O) with known concentrations (see Figure 2.4).

The water content of the homogenized soil samples were also determined. Approximately 10 g of soil was taken from each depth and air-dried for up to 72 hours, until constant weight. The samples were re-weighed and the amount of water was calculated by subtracting the dry weight from the wet weight.

2.2.2. In-Situ Redox Potential Measurements

Redox potential (Eh) measurements were taken in all fields in 1993, 1994 and 1995. Eh was measured by using a platinum electrode, constructed at Louisiana State University Center for Wetland Research, and a standard calomel electrode (SCE) as a reference. The measurements were taken by clipping a meter (Fisher Accumet Model 955 Portable pH/mV Temperature Meter) to an electrode placed in the soil with the reference electrode in the soil, as well. Measurements were adjusted to make them relative to the hydrogen electrode by adding +244 mV.

Before use the electrodes were calibrated using a 0.100 M ferrous ammonium sulfate/0.100 M ferric ammonium sulfate/1.00 M sulfuric acid solution, which has a known Eh of +430 mV with an SCE reference electrode at 25 °C (Light, 1972). The calibrating solution was found to
Figure 2.4. Calibration curve of absorbance vs. concentration of ferrous ions using ferrozine as the indicator, with curve fit and regression.
have a linear dependence on temperature from 10-45 °C (see Figure 2.5); one degree in temperature effected a one mV change in the solution. Temperature adjustments were made in the Eh data in the 1995 season only, however, in all seasons calibration of the meter occurred at temperatures close to 25 °C. All electrodes were within 2% of the standard value. The electrodes were cleaned by immersing them first in a 10% hydrogen peroxide solution, followed by detergent, and then deionized water.

In 1993, two electrodes were placed in each field at a 5-cm depth. A 30-cm PVC pipe (with diameter of approximately 5-cm) was placed 5 cm deep in the soil, one quarter of the way from the rice rows, prior to flooding. The electrodes were put into the soil inside the pipe. They were taken out and cleaned once a week.

In 1994, to measure the Eh at different depths, another method was used. Aluminum channels were cut to a desired length. Two were placed across from each other in the soil, one quarter of the way from the rice plant rows, before flooding. Two plexiglass layers with electrodes held between them slid between the channels. Screws through the plexiglass layers ensured that the electrodes stayed in the position they were placed in. Using this device, the Eh could be measured at any desired depths. The depths measured in 1994 were at 0-2.5 cm, 2.5-5 cm, 5-7.5 cm, and 7.5-10 cm. The platinum tip was placed at the middle of the desired depth.

Readings were taken once a week, except for during the drained times in which they were taken twice a day. Approximately 12 electrodes were in the drained fields and eight in the control field. The electrodes were taken out and cleaned at least once every three weeks. Cleaning during this season included rubbing the platinum tips with sand paper. In 1995, Eh
Figure 2.5. The effect of temperature on the Eh of the standardizing solution (0.100M ferrous ammonium sulfate/0.100M ferric ammonium sulfate/1.00M sulfuric acid solution).
measurements were taken in the three Lemont and three Mars fields in China, Texas, at the second depth, 2.5-5 cm. There were three replicates in each field. Measurements were taken weekly throughout most of the field season. In addition, redox measurements were taken at the Rosenberg site. Three replicates of two depths (2.5-5 and 7.5-10 cm) in both the drained and continuously flooded fields were monitored. Readings were taken weekly or bi-weekly throughout the season.

2.2.3. Soil pH Measurement

In all three years, the pH was measured on the homogenized soil taken by the coring method already described. Approximately 10 g of each sample depth was added to a 60 mL plastic bottle. An equivalent weight of deionized water was added, and the bottle was shaken for 45 minutes on a wrist-action shaker to slurry the soil. In 1995, the soil-water slurry was made by using a motorized hand blender. The pH was then analyzed using a combination glass electrode and meter (Altex Zeromatic IV pH meter in 1993 and Fisher Accumet Model 955 Portable pH/mV Temperature Meter in 1994 and 1995). In 1993, the cores were homogenized separately, and in 1994 and in 1995, the cores were homogenized together.

2.3. Laboratory Methane Production Estimations from Field Collected Soil Samples

In 1994, triplicate soil samples were taken from the three Lemont fields at least once a week during the season. The method for sampling is the same as earlier with the exception that the cores were not kept intact
until analysis. Using a procedure first described by Sass et al. (1990), the cores were divided in the field into four depth intervals: 0-2.5 cm, 2-5.5 cm, 5-7.5 cm, and 7.5-10 cm. Then each 2.5 cm segment placed in a separate whole syringe. The syringe was then filled with an approximately equal quantity of field water to give a total of 30 cm$^3$ volume, and all air was purged. The syringes were transported back to the laboratory using the same method as described previously.

In the laboratory, 30 cc of methane-free nitrogen gas was added, and the syringes were shaken to strip all dissolved gases. The purged gases were removed, and the syringe was re-filled with nitrogen and incubated at 29°C. At 3, 6, 18, and 42 hours the syringes were shaken and head space gas samples were analyzed for methane by the above methods. After each analysis, all gases were removed, 30 cc of nitrogen was added and the syringes were shaken and purged once more. They were then filled with nitrogen before incubation.

The methane concentration was found to vary inversely with time and according to the general equation:

\[ \text{[Methane]} = a \text{(time)}^{-b} \]

A linear relationship is produced by taking the log of each side, as follows:

\[ \log[\text{CH}_4] = -b \log(\text{Time}) + \log a \]

where (log a) is the y-intercept and (-b) is the slope.

The log of the methane concentration was plotted against the log of the time (using the mean of the two sampling times, in hours) for the four
times each sample was analyzed. The instantaneous initial rate of methane production was calculated from the y-intercept (which occurs at T=1 as the log (1) = 0). Individual samples having a slope outside the 95% confidence interval calculated for each field were rejected (6% and 3% of total in 1994 and 1995, respectively). The units of methane production are reported in the same units used for methane flux, in mg CH4 m-2 d-1.

2.4. Total Organic Carbon Determinations

The wet combustion method for total carbon measurement was first presented by Allison et al. (1960). A modified version was reported by Nelson and Sommers (1982), and that is the method used here. Figure 2.6 shows the apparatus used. Trap 2 was used to capture the CO2 for titrimetric analysis.

The reagents used follow:

1. Digestion reagent for carbonates: 57 mL of concentrated sulfuric acid (H2SO4) and 92 g of ferrous sulfate heptahydrate (FeSO4•7H2O) in 600 ml of deionized water, cooled, and diluted to 1 L.
2. Digestion acid mixture: 600 mL of concentrated sulfuric acid (H2SO4) and 400 mL of 85% phosphoric acid (H3PO4).
3. Potassium dichromate (K2Cr2O7), reagent grade
4. Potassium iodide (KI) solution, 50%.
5. Silver sulfate (Ag2SO4) solution, saturated.
6. Carbon dioxide (CO2) absorbent, ascarite.
7. Soda lime, 8- to 14-mesh size.
8. Granular zinc, <30-mesh size.
9. Anhydrous magnesium perchlorate [Mg(ClO4)2]
Figure 2.6. Diagram of the apparatus used to measure the total organic carbon in soil samples (Nelson and Sommers, 1982). For the experiments in this study, a modified trap II was used to capture carbon dioxide which is shown in the inset.
Homogenized soil samples were air-dried, ground, and passed through a 0.5 mm sieve to remove large roots and organic material. A known mass of soil (approximately 1.5 g) was added to a 50 mL round-bottom flask. Three mL of the H2SO4-FeSO4 digestion acid was added. This solution degraded any inorganic carbon compounds. The flask was allowed to sit at room temperature for at least 20 minutes, with occasional turning of the flask. Then, the flask was held upright in a heating mantle, and the contents boiled slowly for 1.5 minutes to destroy any remaining carbonates. The flask was rotated during boiling to avoid excessive bubbling.

After cooling, 2.00 g of pulverized K2CrO7 was added to the flask. 25 mL of the digestion acid mixture was added to the funnel above the condenser. The stopcock was then opened, and the acid mixture was allowed to flow into the round-bottom flask. The stopcock was closed to prevent any loss of CO2. The air delivery tube was placed no more than 1 cm into the acid during digestion. The cooling water and air stream were turned on, and the air was allowed to enter the flask at a rate of 2 bubbles/sec. Using a heating mantle, the sample was boiled for a total heating time of 10 minutes, bringing it to boil in the first 3 or 4 minutes.

After this period, the mantle was removed, and aeration occurred as the air stream was increased to 6-8 bubbles/sec. After 10 minutes, the air stream was turned off, the digestion flask was disconnected from the condenser, and the trap was removed.

The trap consisted of a 50 mL test tube filled with 25 ml of 1 N KOH, which had been bubbled through with nitrogen gas to remove CO2. The tube was also filled with glass beads. A disposable glass pipette connected to the other traps by tygon tubing was inserted into the test tube. After the
aeration period, the pipette was removed, and rinsed with deionized water. The test tube was emptied into a 250 Erlenmeyer flask. Five mL of saturated barium chloride (BaCl₂) was added, and several drops of phenolphthalein. The solution was titrated with 1 N HCl.

On each sampling day, a blank, a sample without soil, was also run using the same procedure. Glass beads were added to prevent bumping. The data were calculated from the following formula:

\[
\text{Total Organic Carbon, \% } = \frac{([\text{ml HCl(blank)} - \text{ml HCl(sample)}]/\text{g soil}) \times \text{N HCl}}{0.6}
\]

where 0.6 is a factor which converts the number of mmoles of HCl not needed for the sample (calculated as the difference between the mL of HCl times the normality of the acid) to the mass percentage of carbon.

2.5. Soil Metals Analysis

Soils from the three fields used in 1994 (Lemont north, center, and south) were taken from the flux boxes. Each field had two flux boxes, and the soils were taken using a 0.2x0.2 m² metal sampling frame. The soils from each frame were divided in two equal parts, and each was divided into four one-inch depth intervals. One half of the soil from each depth was retained intact for most probable number analysis described in the next section. The other half was dried and ground, and then passed through a 0.5 mm sieve to remove root and organic matter. The dried samples were then shipped to the Gulf Coast Waste Disposal Authority for metals analysis.
using plasma emission spectroscopy. The analysis detected concentrations of 25 different metals in the soils.

2.6. Most Probable Number Analysis

Soils taken from the flux frames in 1994 were used for this analysis. As described in the previous section, a portion of each soil sample was retained by placing them intact in plastic bags and kept at 0 °C until analysis.

The growth medium used was Medium WR 86 first described by Fedorak and Hrudey (1986). The ingredients are as follows:

Mineral solution #1
- 50 g/L of sodium chloride (NaCl)
- 50 g/L of ammonium chloride (NH₄Cl)
- 10 g/L of calcium chloride dihydrate (CaCl₂·2H₂O)
- 10 g/L of magnesium chloride hexahydrate (MgCl₂·6H₂O)

Mineral solution #2
- 10 g/L of ammonium molybdate (VI) tetrahydrate
  [(NH₄)₆Mo₇O₂₄·4H₂O]
- 0.1 g/L of zinc sulfate heptahydrate (ZnSO₄·7H₂O)
- 0.3 g/L of boric acid (H₃BO₃)
- 1.5 g/L of iron (II) chloride tetrahydrate (FeCl₂·4H₂O)
- 10 g/L of cobalt (II) chloride hexahydrate (CoCl₂·6H₂O)
- 0.03 g/L of manganese (II) chloride tetrahydrate
  (MnCl₂·4H₂O)
- 0.03 g/L of nickel (II) chloride hexahydrate (NiCl₂·6H₂O)
- 0.1 g/L of aluminum potassium sulfate dodecahydrate
  [Al₅K(SO₄)₂·12H₂O]

Vitamin B solution
- 0.1 g/L of nicotinic acid
- 0.1 g/L of cyanocobalamine
- 0.05 g/L of thiamine
- 0.05 g/L of p-Aminobenzoic acid
-0.25 g/L of pyridoxine
-0.025 g/L of pantothenic acid

Phosphate Solution
-50 g/L of potassium phosphate dibasic (KH₂PO₄)

All of the above solutions were made with ultra-pure water (18 ohm), except for mineral solution #1, which was made using 0.01 M HCl.

Final Composition of Medium WR86
-1.0 mL of Mineral Solution 1
-0.1 mL of Mineral Solution 2
-0.1 mL of Vitamin B Solution
-1.0 mL of Phosphate Solution
-1.0 mL of Resazurin (0.01%)
-0.57 g Sodium Bicarbonate (Na₂CO₃)
-97 mL of Distilled Water
-1.0 mL of Sodium Sulfide (2.5% Na₂S*9H₂O)

All of the ingredients were combined except for the sulfide solution and bicarbonate. The solution was brought to a boil, and continued for two minutes. This was then cooled to room temperature, and the bicarbonate was added. At this time, acetate was also added to produce a concentration of 80 mM (Jain et al., 1991). A CO₂/N₂ gas mixture (20/80) was bubbled through until the pH was 6.9-7.1. After the desired pH was reached, the headspace of the container was continually fed the CO₂/N₂ mixture to ensure anaerobic conditions.

In order to establish anaerobic conditions while the media was placed into the tubes, each tube (Hungate tube, 18 mL) was flushed for 30 seconds (flow rate of approximately 70 mL/min) with the CO₂/N₂ mixture. Then, 9 mL of the media was pipetted into the tube, which was then flushed for
another 30 seconds with the gas. The tube was then stoppered and capped. In a separate tube, the sodium sulfide was added in a similar manner.

A known volume (0.1 mL) of the sulfide solution was then added to each of the tubes. It took approximately five minutes for the sulfide solution to reduce the media, which was witnessed by the change in color of the solution from blue to pink to clear. Resazurin was the indicator used, and turned pink if the Eh was higher than -42 mV (Jain et al., 1991). The tubes were then autoclaved for 20 minutes.

Ten replicates of each dilution were made for each soil sample analyzed. Ten additional tubes were made to which no soil was added. These served as the controls.

Approximately 25 g of soil from each depth of the refrigerated soil (four 2.5 cm depth intervals) were mixed together using a wire pastry blender. Then, 10 g of wet soil was added to 90 mL of a phosphate buffer solution whose components follow:

Phosphate Buffer Solution
-17 g/L of potassium phosphate dibasic (KH₂PO₄)
-43.5 g/L of potassium phosphate monobasic (K₂HPO₄)
-66.8 g/L of sodium phosphate monobasic (Na₂HPO₄)

This was then mixed with a hand held blender until the soil particles were dispersed. During the mixing, the CO₂/N₂ gas mixture was bubbled through the solution. Bubbling was allowed to continue for 10 minutes after mixing. Then, approximately 1 mL of the soil/buffer solution slurry was transferred to the tubes, which represented a 10⁻¹ dilution. A series of dilutions were made, through 10⁻⁴ for all fields. The ranges for the
dilutions were based on preliminary experiments of dilutions ranging from $10^{-1}$ to $10^{-10}$ carried out with both south and north field soils.

The tubes were placed in an incubator set at approximately 37 °C for a period of 6 weeks. Earlier studies were conducted for a time period of only 4 weeks, however, acetotrophic methanogens are well known for their slow growth rate, and so the incubation time was increased (Jain et al., 1991). The tubes were manually shaken intermittently throughout their incubation period (approximately one to two times per week). After incubation, the tubes were taken out of the incubator, and their headspace was analyzed for CH4. The gas was analyzed using a gas chromatograph fitted with a flame ionization detector (Shimadzu GC-8AIF).

If the inoculated sample had a methane concentration higher than the control, it was counted as a positive sample. If it was the same or lower than the control, it was counted as a negative sample. Using the number of positive samples and an MPN table, the concentration of methanogens in the soil could be determined (Koch, 1981).

The actual number was found by using only three dilutions, starting with the largest concentration that has all ten tubes positive. By counting the number of positive samples for the next two dilutions, three numbers were gathered for each soil sample. These numbers were easily translated into an MPN index/100 mL which is then used in the following formula for the final number:

\[
\text{MPN/middle dilution} = \text{MPN bacterial count}
\]

2.7. Root Distribution
In 1994, four syringe cores were taken one quarter of the way from the rice plant for root distribution. These were taken 5 times over the season, on days 14, 29, 42, 57, and 73 DAF in both the north and south fields. After being transported to the laboratory, the samples were frozen until analysis.

After thawing, the soil cores were removed from the syringes and divided into four 2.5 cm increments. The soils were placed in small plastic bags to which a small amount of water was added. The soil-water mixture was then mixed by hand to disperse the soil. Then the bags were emptied into 0.5 mm sieves and rinsed with water. The roots were removed from the sieve (both dead and live) and placed in a container for drying. Drying occurred over 48 hours in an oven set at approximately 70 °C. The dry roots and paper were weighed then scraped off the paper, and the paper was re-weighed. The weight of the roots was calculated by subtracting the paper weight from the weight of both the roots and the paper.

2.8. Algal Mat Development

In 1994, the algal mat development was observed in the north and south fields in both Lemont and the non-rice fields. Following a design by my advisor, Dr. Frank Fisher, an apparatus was built which was placed in the soil and monitored the growth of the algal mat on the soil surface. The device consisted of a plexiglass rectangle approximately 10 inches long and 6 inches wide. At the top of the rectangle were holes through which metal bars were attached with screws. The metal bars were used to hold some light weight mesh cloth that was folded around the bottom of the rectangle.
The cloth would serve as a physical substrate for the algal mat in the soil. These were buried near 30 DAF and removed at the end of the season.

2.9. Outdoor Pot Experiment

An outdoor pot experiment was conducted in 1994 on the campus of Rice University. Soil was collected from China, Texas, in a field adjacent to the north field used in the 1994 and 1995 seasons. Soil was passed through an 8 mm sieve to remove organic matter, and then mixed to ensure homogeneity. Seeds of the rice cultivar Lemont were planted on July 22 in 25 cm diameter and 22 cm high plastic pots (approximately 5 seeds per pot). Flooding occurred on August 22, 1994. Flooding was accomplished by placing the pots randomly into wading pools approximately 28 cm high. A floodwater height of 6 cm was maintained.

Water in the pools was standard tap water which had been allowed to stand in storage pools outside for several days for passive dechlorination. Holes were cut into the sides of the pot above the soil line for movement of water between the pool and the pots.

Transplanting directly prior to flooding ensured each pot had an equal number of tillers (7-11). Fertilization of nitrogen and phosphorus, in equal amounts, occurred on August 3 (40 kg N and P ha\(^{-1}\)), August 24 (50 kg ha\(^{-1}\)), and October 10 (50 kg ha\(^{-1}\)), 1994.

Pots were organized in sets of five for each treatment. Each set was monitored for methane flux and redox potential. A summary of treatments follow:

- Control vegetated
- Control bare
- Drained vegetated

The drained pots were removed from the pool on October 4 (43 DAF). They were left drained until October 7 (46 DAF), at which time they were placed back into the pools and re-flooded.

Methane flux measurements were taken weekly on the control vegetated and drained vegetated sets and every other week in the bare control set. Cylindrical flux chambers were made from five gallon plastic water bottles fitted with plastic collars which went over the upper rim of the pots by approximately five cm. The collars were well below the water surface, and this inhibited the flow of air in or out of the chamber while fluxes were being taken. The chambers were covered with aluminum foil to reduce warming inside.

Flux samples were taken in sets of five pots in random order. The average chamber height was approximately 70 cm. A stopper fitted with a copper tube in the middle was placed in the top of the chamber at time zero. Tygon tubing attached a syringe to the copper tube, and 60 mL samples were taken every five minutes. Before sampling, the syringes were pumped vigorously for 30 seconds to ensure mixing within the chamber.

Gas samples were collected and analyzed as in the field. This includes the measurement of ambient air temperature, soil and water temperatures, temperature within the flux chamber, water depth, and plant height. Methane mixing rations were determined with a gas chromatograph fitted with a flame ionization detector (Shimadzu GC-8AIF). CH₄ emission was determined from the slope of the mixing ratio change in each set of five samples. Plots of methane concentration versus time that did not yield a
linear regression value of $R^2$ greater than 0.90 were rejected. Units of methane emission are $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$.

Platinum electrodes were used as in the field for measurement of Eh. The electrodes were placed 6.25 cm into the soil, usually in replicates of two. They were measured at most twice a day, and at least three times per week. Readings were taken as in the field.
3. RESULTS OF SEASONAL TRENDS

Seasonal soil redox trends in submerged soils are well documented (Ponnamperuma, 1972). Seasonal patterns in Texas rice paddies were not as well known. Therefore, extensive measurements of soil redox conditions in Texas rice paddies were taken over four years, 1992-1995. Preliminary ferrous ion concentrations and pH values from the first season were presented previously (Lewis, 1994). Results here report measurements of methane production and various redox parameters throughout these consecutive seasons in different field sites in the cultivar Lemont. They are then contrasted with trends found in other sites.

3.1. Eh Results

As stated in Chapter 1, the reduction potential of soils, normally +400 to +700 mV in aerated soils, tends to decrease upon flooding (Patrick, 1981). Upon flooding, the Eh decreases sharply to negative values, in the range of -100 to -300 mV. The decrease in Eh is due to a series of other electron acceptors being utilized after oxygen by a succession of facultative and obligate anaerobic bacteria.

Daily seasonal Eh trends in Texas rice paddies follow the generally reported pattern upon flooding. Decreases occurred immediately with steady negative values being reached between 20 and 30 DAF (days after flooding) (See Figure 3.1). In Figure 3.1, the redox potentials were first averaged over all depths monitored and over replicate fields for each year, including an outdoor potted rice study. Then, these values were plotted against time. In 1994, two fields (north and south) underwent two drains.
Figure 3.1. The average daily Eh values for four different seasons plotted against time. The error bars are standard deviations.
The effect of these drains is removed from Figure 3.1 and all subsequent graphs in this Chapter so that general trends from that year can be compared with other years which did not experience drainage. This was accomplished by using only data from the control field for the time during the season when the drainage effects were most obvious, 64 DAF and following. Also, extrapolation in the early season using data from the north and south fields was necessary, since data was not gathered from the control field until 21 DAF. However, at that point of the season, the three fields had very similar results in all parameters measured.

The agreement between the different years is strong, with the exception of the 1993 season. The values from 1993 are more positive than those of the other years during the middle of the season (15-40 DAF). With the exception of 1993, overlap of the remaining fields is visible until day 30, at which time the average Eh was -90 mV (±59, SD). After day 30, there were no significant differences among any of the fields.

Eh data to this point have been presented as an average of all depths measured. In the 1994 field study, electrode measurements were taken at four 2.5 cm increments throughout the season in three different fields (north, center, and south). Data is presented here for the center field (see Figure 3.2a). Upon flooding, the soil closest to the surface appeared to be affected more quickly than the lower depths. The top 2.5 cm dropped in Eh first, and then the second 2.5 cm dropped, which was followed by the third. The bottom depths did not reach similar values until near 51 DAF. After day 51, there were no differences among the depths.

In 1995, the cultivar Lemont was grown in another location in Rosenberg, Texas and the Eh was monitored at two depths: 2.5-5 and 7.5-10 cm (see Figure 3.2b). The differences between the depths in this field
Figure 3.2. The redox potential plotted by depth against time for (a) Lemont in China, Texas, 1994, (b) Lemont in Rosenberg, Texas 1995. Error bars are standard deviations.
are similar to those of the center field of 1994. The Eh of the higher depth dropped below -100 mV sooner than the bottom, around 30 DAF. The bottom depth did not reach similar negative values until 50 DAF. After that point, both depths had similar Eh values.

3.2. Ferrous Ion Concentration Results

The concentration of ferrous ions in submerged soil has been found to increase to values as high as 600 ppm within 1-3 weeks after flooding and then experience an exponential decrease to 100-300 ppm which remains for several months (Ponnaiperuma, 1981; Ponnaiperuma, 1972). Patrick (1981) showed ferrous ion concentration increasing to values greater than 2000 ppm after flooding in paddy fields.

The results from Texas rice paddies indicate similar increases at the beginning of the season, although no further decrease occurred later in the season. Results were averaged over all depths for the 1993 and 1994 seasons for the cultivar Lemont (see Figure 3.3). The seasonal trends for the two years are very similar. The seasons were divided into two parts: a period of dramatic increase until day 30 followed by a slower increase to the end of the season. In 1993, increases were monitored as early as from 2 DAF. In 1994, a slightly slower rate of increase was observed beginning near 7 DAF.

In comparing the ferrous ion concentrations in different soil depths, the center field measurements from 1994 were plotted against time. It is apparent that the lowest depth was least affected by the flooding, not reaching values similar to the other depths until 51 DAF (see Figure 3.4),
Figure 3.3. The average daily ferrous ion concentration for two different seasons, 1993 and 1994, plotted against time. Error bars are standard deviations.
Figure 3.4. The ferrous ion concentration of Lemont Central, China, 1994, plotted by depth against time. Error bars are standard deviations.
which agrees with the Eh results presented in the previous section (see Figure 3.3).

3.3. Results of pH

The soil pH seasonal trend upon flooding is well-known (Ponnampalam, 1972). The pH in acidic soils tends to increase toward neutrality, and the pH of basic soils tends to decrease. Results from Texas rice paddies show the same general trend as predicted for acid soils (see Figure 3.5). These data are comparable with results from a study in 1992 in similar locations (Lewis, 1994).

In both 1993 and 1994, pH values were similar early and late in the season. Both years experienced an increase until 30 DAF. In 1993, the maximum pH at 30 DAF was 5.90, and the remained at similar levels for the remainder of the season. In 1994, a sharper increase occurred to a maximum of 6.70, which was followed by a decline and leveling off to values similar to those earlier in the season.

Depth differences occurred in pH measurements (see Figure 3.6). The top depth showed the most rapid response to flooding in 1994, followed by each successive depth (see Figure 3.6a). Whereas the top depths experienced an increase to 30 DAF followed by a decrease thereafter, the bottom 2.5 cm only increased throughout the season. A one-way analysis of variance test showed that after day 29 the depths were significantly different (p<0.01). A Tukey’s post-hoc test showed that the two top depths were significantly different from the bottom two, however, there were no differences between the two top depths or between the two
Figure 3.5. The average daily pH of two different seasons plotted against time.
Figure 3.6. The soil pH by depth plotted against time for (a) Lemont Central, China, Texas, 1994, and (b) Lemont, China, Texas, 1993.
bottom depths. The average pH values after day 29 were 5.89 (±0.19, SD) and 6.17 (±0.13) for the depths 0-5 and 5-10 cm, respectively.

Depth differences in the 1993 data were not as clear or dramatic (see Figure 3.6b). However, the two depths (0-5 and 5-10 cm) appear to have the same general trends as in 1994, although the crossover point in 1993 was much later than in 1994 (after 40 DAF). At this point, the top 5 cm does show a decline in pH while the bottom 5 cm continues to increase. The average values for after day 29 are 5.72 (±0.14, SD) and 5.94 (±0.07) for the depths 0-5 and 5-10 cm, respectively. After day 43 DAF, these change to 5.64 (±0.06) and 5.96 (±0.09).

3.4. Methane Production Results

Methane production, or the potential production upon incubation of soil under an atmosphere of nitrogen, tends to increase upon flooding. Its trend has been documented previously (Sass et al., 1992, 1991a, 1991b, 1990). Sass et al. (1992) described an emission:production ratio that decreased throughout the season. This may have been due to an increase in methane oxidation when oxidation is viewed as the difference between production and emission. Percent oxidation was reported as increasing from below 20% at 20 DAF to near 80% by 50 DAF.

Soil cores were taken to measure methane production throughout the 1994 season, and three times during the 1995 season (center field Lemont). Production measurements in 1994 began to increase near 20 DAF until a maximum was reached on day 70. Values decreased until 78 DAF which was followed by a sharp increase from 85-91 DAF (see Figure 3.7).
Figure 3.7. Methane production rates for the 1994 Lemont Central field plotted for all four depths against time.
Though the 1995 methane production rates were much lower than those in 1994, methane production rates still increased throughout the season (see Table 1). All three methane production values in 1995 were 21\% or less than 1994 values, and day 41 had values only 1\% of the 1994 values.

Table 3.1. Comparison of methane production values from 1994 and 1995 with standard deviations given in parenthesis, including the 1995/94 ratio. The units for methane production rates are mg CH4 m\(^{-2}\) d\(^{-1}\).

<table>
<thead>
<tr>
<th>Time, in DAF</th>
<th>1994</th>
<th>1995</th>
<th>95/94 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>38.4 (33.83)</td>
<td>2.7 (0.89)</td>
<td>7%</td>
</tr>
<tr>
<td>41</td>
<td>431.4 (42.13)</td>
<td>2.2 (0.57)</td>
<td>1%</td>
</tr>
<tr>
<td>62</td>
<td>1018.8 (111.39)</td>
<td>215.1 (129.03)</td>
<td>21%</td>
</tr>
</tbody>
</table>

Figure 3.7 also shows the amount of methane produced in each 2.5 cm depth increment. Throughout the season, the highest production throughout the season occurred in the 2.5-5 cm depth. However, late in the season, near 64 DAF, the top depth (0-2.5 cm) began producing at similar high levels. A drop in production on day 67 after flooding may correspond to a partial unscheduled drain that occurred in the central field at that time, which lowered the production rates in the top depth.

In 1995, the only day comparable to 1994 production rates was 62 DAF. At that time, the middle two depths were responsible for the majority of methane production. Production rates for that day were 4.05 (±3.05, SD), 106 (±74.8), 100 (±99.5), and 4.45 (±2.98) mg CH4 m\(^{-2}\) d\(^{-1}\) for depths 0-2.5, 2.5-5, 5-7.5, and 7.5-10 cm, respectively.
4. RESULTS OF FIELD DRAINAGE

Field drainage has been found to be an effective method for the mitigation of methane emissions from rice paddies (Sass et al., 1992; Yagi and Minami, 1992). Field drainage is thought to inhibit the production of methane by the re-entry of air into the reduced soils. The purpose of my research was to investigate the extent of oxidation during a drainage period and the return to reduced conditions following re-flooding. This was accomplished by monitoring the soil redox parameters described previously and methane production rates throughout a mid-season drain and re-flooding events in several fields and an outdoor potted rice study over two different years. The results from these studies will be divided into the overall effects of drainage and variation by depth.

4.1. Overall Results on the Effects of Drainage

Different parameters were measured in the three seasons in which mid-season drainages occurred. Table 4.1 lists the parameters measured during each season. The cultivar used in all seasons was Lemont.

<table>
<thead>
<tr>
<th>Year</th>
<th>Fields Drained</th>
<th>Control Fields</th>
<th>No. of Drains</th>
<th>Eh</th>
<th>[Fe2+]</th>
<th>pH</th>
<th>CH4 Prod</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>yes *</td>
<td>yes *</td>
<td>yes *</td>
<td>yes *</td>
</tr>
<tr>
<td>1994</td>
<td>5 pots</td>
<td>5 pots</td>
<td>1</td>
<td>yes**</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>1995</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>yes ^</td>
<td>no</td>
<td>yes ^</td>
<td>no</td>
</tr>
</tbody>
</table>

*all four 2.5 cm increments, **5-7.5 cm, ^2.5-5 and 7.5-10 cm

Methane production rates, Eh, pH and ferrous ion concentrations were measured throughout the 1994 season. Two fields, north and south,
were drained, and one field was used as a continually flooded control, the center field. Their results are shown for the 2.5-5 cm depth for all fields, including extrapolation for the early season for the control field as explained in Chapter 3 (see figures 4.1a-d).

In Figure 4.1a, methane production rates are shown. The drained fields exhibited a seasonal pattern similar to the control field until drainage periods, which caused decreases in methane production rates as found in other studies (Sass et al., 1992, Yagi et al., 1992). The first drain, between days 30 and 35, resulted in a decrease of 83% in the north field with negligible changes in the south field. Rebounding of methane production in the north field occurred within one week following re-flooding. This drain’s effect may have been minimized by heavy rainfall and lower temperatures that occurred during the drain.

The second drain occurred between days 64 and 69. Weather during this drain was clear and without rain. Unlike the first drain, the second drain caused the methane production to drop and remain at negligible levels for up to three weeks after re-flooding. In comparing the pre-drain values to those three weeks after re-flooding, production rates at the later date were only 4% (±13) and 37% (±10) of the earlier date for the north and south fields, respectively, and 135% (±15) in the center, undrained field. The drained fields never reached levels comparable to pre-drain values.

Drainage has been found to cause in increase in Eh (Yagi et al., 1992; Faulkner, 1989). Yagi et al. (1992) reported an increase in Eh at a 2 cm depth of up to 800 mV upon draining. In this study, the first partial drain resulted in an increase by 140 mV to a value of 8.5 mV on day 35 in the north field (see Figure 4.1b). The Eh rebounded back to pre-drain
Figure 4.1. Results from the 1994 season vs. time for (a) methane production rates, (b) Eh, (c) ferrous ion concentration, and (d) pH. Data are shown for the north, south, and center fields. The 2.5-5 cm depth increment was used for figures b-d. Drainage events are shown with dashed lines.
levels (-26 mV, day 29) within one week following re-flooding. The Eh values for the south field during this time were not different than the control field. The second drain caused a much larger increase of 431 mV to a value of 334 mV on day 69. The south field showed an increase of 571 mV during the second drain. This drain had a stronger effect on Eh and 20 days after re-flooding, the Eh was still positive in both fields.

Drainage has been found to cause a decrease in the ferrous ion concentration (Lewis, 1994). This may be due to the re-entry of oxygen at drainage and the subsequent oxidation of soluble ferrous ions to ferric complexes. In the north field, the first drain caused a decrease in concentration of 401 ppm, while the second drain caused a decrease of 1941 ppm (See Figure 4.1c). The south field experienced decreases of 710 ppm and 2967 ppm in the first and second drainages, respectively. In both fields, the first drain rebounded within 15 days after re-flooding. The recovery after the second drain was slower. Concentrations were 328 ppm lower than pre-drain values in the north field and 1328 ppm lower in the south field 21 days after re-flooding.

Whereas flooding causes an increase in the pH of acid soils, drainage causes a decrease to occur (Hanif et al., 1986). The pH of the three 1994 fields is shown in Figure 4.1d. There was no detectable decrease in pH that could be associated with the first drain. However, at that time, all three fields exhibited a drop which may be due to the increase in organic acids and CO2. The second drain, significantly affected the north and south fields, and it caused a drop to pH values similar to or lower than those at the beginning of the season.

In 1994, the methane flux and Eh were also measured in an outdoor pot study which underwent one mid-season drain (see Figure 4.2). Both
Figure 4.2. Results from the 1994 outdoor potted rice study for (a) methane emission rates and (b) Eh vs. time. Dashed lines represent drainage events.
the control and drain sets had methane emission rates which began to increase near 15 DAF (see Figure 4.2a). A maximum was reached on day 45 for the control pots (193 ± 39 SD mg CH4 m^-2 d^-1). The drained pots showed a maximum on day 42 (143 ± 15 SD mg CH4 m^-2 d^-1) dropping to negligible amounts after draining on day 43. A decrease (46%) in the control pots also was detected during the drainage period. This may be due to a decrease in soil temperature of up to 4 °C during that time. The fluxes in the drained pots did not recover, as sampling 15 days after the re-flooding still reported negligible amounts of methane.

The Eh in depth 5-7.5 cm showed a response to flooding as previously described by Ponnamperuma (1981). There was a rapid drop in Eh near day 7 followed by a rise and then a slow decrease to a minimum (-98±16, SD) that remained throughout the season for the control pots (see Figure 4.2b). Drainage occurred on days 43-46 after flooding. The drained pots showed an increase in Eh of 472 mV to a value of 179.6 (±54.7 SD) mV on day 48. The Eh in the drained pots was still positive (80 ± 60 SD mV) 12 days later.

In 1995, the last drainage study was performed in a different soil type in Rosenberg, Texas. In this field, Eh and pH values were measured in control and drained fields. The Eh showed trends similar to those already presented. Eh in both the control and drained field decreased after flooding (see Figure 4.3a). A minimum was reached in Eh by 50 DAF. The Eh values of both fields at this time were -201 (±3) and -195 (±4) mV for the control and drained field, respectively.

A drain occurred from 50 DAF to 56 DAF. Between 50 and 55 DAF, the Eh in the drained field jumped 455 mV to a value of 291 (±38) mV while the flooded field was still negative at -174 (±4) mV on day 55.
Figure 4.3. Results from the 1995 Rosenberg, Texas, study showing the (a) Eh and (b) pH vs. time. Data are shown for the 2.5-5 cm soil increment. Drainage times are marked with dashed lines.
After re-flooding on day 56, the Eh of the drained field dropped steadily until 22 days later, on 78 DAF, the value was similar to the Eh of the flooded field. The Eh values on 78 DAF were -167 (±7) and -159 (±8) for the flooded and drained field, respectively.

The pH trends of the Rosenberg fields were similar to those of the fields in the 1994 China, Texas, study. Upon flooding, the pH increased, and during drainage events the pH decreased (see Figure 4.3b). Early in the season, the control field had a dip in pH close to 10 DAF which may correspond to the partial drain which occurred in that field at that time.

The drainage that occurred between 50 and 56 DAF resulted in a drop of 1.64 pH units in the drained field. Re-flooding caused an increase in pH to a final value of 7.02 (±0.01) on 78 DAF, which was slightly higher than the control field. The control field remained unaffected during the drain event, with a seasonal maximum on 78 DAF of 6.88 (±0.02).

4.2. Variation by Depth During the Drain

Redox parameters were monitored over several depths in order to gain information about the gradual introduction of air into previously reduced soils upon field drainage. In the 1994 China, Texas, study, the redox potential, methane production, and the ferrous ion concentration were measured during the drain at four 2.5 cm depths. These results are presented here at the following three intervals: 0-2.5 cm, 2.5-5 cm, and 5-10 cm. In the 1995 Rosenberg study, only two depths were studied - 2.5-5 and 7.5-10 cm. The China, Texas, results will be discussed first.
Table 4.2. Significance levels for the Eh, ferrous ion concentration, and methane production trends by depth and over time for the north and south fields during field drainage, as calculated by a two-way ANOVA test.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Field</th>
<th>p (Depth)</th>
<th>p (Time)</th>
<th>p (Depth*Time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eh</td>
<td>North</td>
<td>0.002</td>
<td>0.001</td>
<td>0.243</td>
</tr>
<tr>
<td></td>
<td>South</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>[Fe2+]</td>
<td>North</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>South</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>CH4 Production</td>
<td>North</td>
<td>0.233</td>
<td>0.000</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>South</td>
<td>0.933</td>
<td>0.001</td>
<td>0.942</td>
</tr>
</tbody>
</table>

Results show that Eh varied by depth during the drainage period (see Figure 4.4). It appears that the top depths react more quickly and dramatically to the drain compared to the bottom five cm. Two-way repeated measures analysis of variance tests were calculated for both fields and confirm this (see Table 4.2). Eh was significantly different between the sampling times and between the depths. There was a significant interaction for the south field only. One way analysis of variance tests done for both the south and north field over the three depths and at each day during the drain show that before the drain and for the first day following, the depths were not significantly different (see Table 4.3). Both fields show that the top 2.5 cm was significantly different than bottom 5 cm by the third day after draining (67 DAF). By the fifth day after draining, both the 0-2.5 and 2.5-5 cm intervals were significantly different than the bottom 5 cm. One day after re-flooding, there were no significant differences between any of the depths in the south field. In the north field, both of the top depths were different from the bottom 5 cm.
Figure 4.4. The Eh of the north and south fields by depth during the drain in 1994. Error bars are standard deviations. Drainage occurred after sampling on 64 DAF, and reflooding occurred after sampling on 69 DAF.
Table 4.3. Significance levels for the Eh and ferrous ion concentration for the north and south fields, as calculated with the Tukey HSD post-hoc test.¹

<table>
<thead>
<tr>
<th>Time (DAF)</th>
<th>Depth Comparison²</th>
<th>p (Eh-N)</th>
<th>p (Eh-S)</th>
<th>p ([Fe2+]N)</th>
<th>p ([Fe2+]S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>1-2</td>
<td>*</td>
<td>*</td>
<td>0.987</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>*</td>
<td>*</td>
<td>0.066</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>*</td>
<td>*</td>
<td>0.050</td>
<td>*</td>
</tr>
<tr>
<td>65</td>
<td>1-2</td>
<td>*</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>*</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>*</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>66</td>
<td>1-2</td>
<td>*</td>
<td>0.004</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>*</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>*</td>
<td>0.309</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>67</td>
<td>1-2</td>
<td>0.271</td>
<td>0.016</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>0.002</td>
<td>0.002</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>0.16</td>
<td>0.097</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>69</td>
<td>1-2</td>
<td>0.196</td>
<td>0.863</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>0.004</td>
<td>0.059</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>0.039</td>
<td>0.038</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>70</td>
<td>1-2</td>
<td>0.251</td>
<td>*</td>
<td>0.162</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>0.006</td>
<td>*</td>
<td>0.000</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>0.049</td>
<td>*</td>
<td>0.002</td>
<td>0.019</td>
</tr>
</tbody>
</table>

¹ The Tukey test was used on dates which were found to have a p<0.05 using a one-way ANOVA test. ² Depths represent the following increments: 0-2.5 cm (1), 2.5-5 cm (2), and 5-10 cm (3). *depths were not found to be significantly different using one-way ANOVA test. (-) data were not collected at that time.

The ferrous ion concentration also showed variation by depth with the drain (see Figure 4.5). Two-way analysis of variance tests reveal significant differences between the depths and over time, as well as significant interactions for both the north and south fields (see Table 4.2). The top 2.5 cm appeared to drop first, followed by the second 2.5 cm, and the bottom 5 cm did not change until after day 67. The day following re-flooding all three depths exhibited their minimum ferrous ion
Figure 4.5. Ferrous ion concentration plotted against time during the drain for the north and south fields. Error bars are standard deviations. Drainage occurred after sampling on 64 DAF and reflooding occurred on 69 DAF.
concentration. One way analysis of variance tests results show that the north field had significant differences between the top two depths and the bottom 5 cm before the drain (see table 4.3). Three days after the drain in both fields, all three depths were different from each other. Following re-flooding, the top two depths were different from the bottom in both fields.

The methane production results also differed throughout time, however, differences by depth were not evident (see Figure 4.6). Any depth differences were not significant compared to the large natural variance in the samples (see Table 4.2). Both fields showed differences with time, however, and post-hoc tests reveal the pre-drain to be significantly higher than days 67 and 70.

The Rosenberg field data showed similar overall patterns by depth for Eh and pH. The data is shown for the two depths measured: 2.5-5 and 7.5-10 cm. Eh data reveal that by day 50 in the flooded field, both the upper and lower depths were similarly negative (see Figure 4.7). The drained field, however, was drained near 50 DAF, and thus the bottom depth never reached similar negative values to the upper depth. Three days after draining, at 53 DAF, the upper depth had reached values near 300 mV. Five days after draining, at 55 DAF, the upper depth maintained its positive Eh readings. The lower depth experienced no change throughout the drain, instead it maintained pre-drain values. It may be that the drain inhibited a decrease in Eh values seen at that time in the season, rather than increasing the Eh in the bottom depth. On 55 DAF, however, there was an increase in the standard deviation of the Eh of the bottom depth, which makes it appear that a transition to more positive Eh values may be beginning to occur. The three Eh data points for the bottom depth on 55 DAF were 30, -151, and 203 mV for an average of 27 (±177) mV. This
Figure 4.6. Methane production plotted against time during the drain for north and south fields. Error bars are standard deviations. Drainage occurred after sampling on 64 DAF, and reflooding occurred on 69 DAF.
Figure 4.7. Eh by depth for the (a) control and (b) drained fields in Rosenberg, Texas, 1995. Error bars are standard deviations. Effective drainage dates are 50-56 DAF.
wide range of values is indicative of a system that is widely heterogeneous and supporting a vast range of intermediates between a soil that is neither completely reduced or oxidized.

Re-flooding occurred on 56 DAF, and by 60 DAF the Eh of the upper depth had fallen to 64 mV (±66) in the drained field. By this time, the Eh of the lower depth had actually increased to an overall value of 75 mV (±50). The readings of the upper and lower depths remained similar and positive on 64 DAF. Measurements on 78 DAF, 22 days after re-flooding, revealed that the Eh of the upper depth had decreased again to -159 (±8) mV. This suggests the re-establishment of anaerobic conditions. The large error associated with the lower depth at that time may be indicating that a transition is beginning to occur from oxidized to reduced conditions.

The pH by depth results are supportive of the data from China, Texas, presented earlier (see Figure 4.8). In the control field, the pH gradually climbed to a maximum near 43 DAF at a value close to 7 and stayed there for the remainder of the season. The decline in pH on 12 DAF may be due to a partial drain in that field as previously described. In the drained field, there was also a maximum on 43 DAF, however, there was not a steady climb to the maximum as in the control field.

Three days after draining, on 53 DAF, the pH of the upper depth plummeted 1.77 pH units and remained low until re-flooding. Rebounding of the pH occurred within four days after re-flooding, with pH values 0.6 units higher than the minimum on 53 DAF. The pH continued to rise after re-flooding to a maximum on 78 DAF of 7.01. The lower depth also decreased throughout the drain, however, it was characterized by a slower decline of 0.65 pH units from pre-drain to re-flooding (43-55). Like
Figure 4.8. The pH of the soil by depth for the Rosenberg (a) continually flooded and (b) drained fields. Effective drainage dates are 50-56 DAF.
the upper depth, rebounding occurred quickly in the lower depth. By 5 days after re-flooding, 64 DAF, the pH of the lower depth was higher than pre-drain values. Additionally, by 78 DAF, pH values in both depths were similar in the control and drained fields. Differences of 0.125 and 0.06 were evident for the upper and lower depths, respectively, with higher values in the drained field.
5. RESULTS OF CULTIVAR DIFFERENCES

An important mitigation option for controlling methane emissions from rice paddies is the use of different rice cultivars. Previous studies have shown that different wetland plants emit different amounts of methane gas (Whiting and Chanton, 1993; Barber et al., 1988). In 1993-95, studies were conducted with different rice cultivars. Methane seasonal emission in 1993 varied from 18-41 g m\(^{-2}\) among ten cultivars. Redox parameters were measured in these fields to determine if there were any variation in the reduction processes among the cultivars. Two different cultivars were monitored in the 1995 season. Studies were done to compare reduction processes in cultivar and non-rice plots in 1993, 1994 (outdoor pot), and 1995 seasons.

5.1. Differences Between Rice and Non-Rice Plots

The Eh, ferrous ion concentration, pH, and TOC were measured in both rice (cultivar Lemont) and non-rice plots over several seasons. The Eh will be considered first. In 1993, Eh readings were taken at approximately 5 cm below the soil surface. In the 1994 outdoor potted rice studies, electrodes were inserted approximately 6 cm below the soil surface. In 1995, Eh readings were taken in the middle of 2.5-5 cm (3.25 cm) below the soil surface.

There were significant differences in Eh between the two plots among the three years. In 1993, there were no significant differences between Lemont and the non-vegetated plot (see Figure 5.1a). However, in both 1994 and 1995, the Eh in the non-rice plots were more positive than
Figure 5.1. The Eh of Lemont and non-rice plots for (a) 1993, (b) 1994 outdoor pot, and (c) 1995 seasons. Error bars are standard deviations.
the Eh in the rice plots. In 1994, the Eh values of the rice and non-rice pots were similar only early in the season (until 20 DAF) (see Figure 5.1b). At that point the rice pots showed an additional decrease to values near -100 mV, however, the bare pots hovered near 0 mV until 40 DAF at which time it began a gradual increase to 100 mV where it remained throughout the season.

Similarly in 1995, after 10 DAF, redox values differed between the two plots (see Figure 5.1c). In that year, the Eh values in the non-rice areas never went below 0 mV, but increased to 400 mV near 30 DAF. They remained at high levels throughout the remainder of the season.

The ferrous ion concentration was also measured in both Lemont and non-rice plots in the 1993 season. Data from two different 5 cm increment depths (0-5 and 5-10 cm) were compared (see Figure 5.2a). A paired t-test showed no significant differences between rice and non-rice areas (p>0.05). Both plots have a general increase in the ferrous ion concentration throughout the season.

Unlike the ferrous ion concentration, there were pH differences between the two plots. From 1993 data, the non-rice plot had consistently higher pH values than the cultivar (see Figure 5.2b). In a paired t-test, the top 5 cm was significantly different than the Lemont plot. The grand means for the top 5 cm of the bare and cultivar plots are 6.00 and 5.77, respectively.

In addition to redox parameters, total organic carbon (TOC) in the soil was also monitored throughout the 1993 season in rice and non-rice

---

1The rice cultivars compared to the non-rice plot did not include the cultivar Lemont, but were cultivars labelled 7-10 (see figure 2.1). As will be shown in the next section the ferrous ion concentration of Lemont was not significantly different from the ferrous ion concentration of cultivar group 7-10. However, the division of cultivars is necessary due to different sampling schedules used for cultivars 1-6 and 7-10.
Figure 5.2. Results for both five cm soil increments in Lemont and non-rice plots of the (a) ferrous ion concentration, (b) pH and (c) % TOC. The pH and %TOC in the top depth were significantly different between the two fields (p<0.05).
plots. TOC values were not found to change significantly over time in any field measured (see Figure 5.2c).

Since, no differences were detected over the course of the season, TOC values were averaged over time in order to compare cultivar and non-rice plots. Table 5.1 lists the average percent TOC of all cultivars, Lemont only, and the non-rice plot. A significant difference was found between the Lemont and non-rice plot in the top depth (p<0.01). However, there appeared to be no difference between all ten cultivars and the non-rice plot.

Table 5.1. The average percent total organic carbon measured in two depths in a non-rice plot, Lemont only, and all cultivars during the 1993 field season. Data were collected in two 5 cm depths, and the standard deviations are given in parenthesis.

<table>
<thead>
<tr>
<th>Field/Depth</th>
<th>0-5 cm</th>
<th>5-10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-rice</td>
<td>1.45 (0.21)</td>
<td>1.31 (0.21)</td>
</tr>
<tr>
<td>Lemont</td>
<td>1.21 (0.23)</td>
<td>1.10 (0.18)</td>
</tr>
<tr>
<td>All cultivars</td>
<td>1.33 (0.19)</td>
<td>1.22 (0.20)</td>
</tr>
</tbody>
</table>

5.2. Cultivar Differences

In 1993, the seasonal Eh was monitored for 10 different cultivars. Statistical tests revealed no differences among the cultivars with time, therefore the Eh measurements from all the rice plots were averaged (see Figure 5.3). The points were fitted with a logarithmic curve (r2=0.932). The standard deviation shown for each sampling date was calculated from the standard deviations of the individual fields. Individual Eh data points are also included to show that no cultivar is consistently more than one standard deviation away from the average.
Figure 5.3. The average Eh of all ten cultivars used in 1993 vs. time. Error bars represent the standard deviations calculated from the standard deviations of the individual cultivars. Points outside one standard deviation from the average are labelled by field position of the cultivar: 1-LeBonnet, 2-Lemont, 3-Dawn, 7-Mars, 9-Labelle, and 10-Jasmine.
The seasonal pattern of a decrease in Eh is visible for all cultivars. The average Eh of all 10 plots was 412 mV two days after flooding. It decreased steadily to a negative value maximum of -124 mV 70 days after flooding.

In 1995, the Eh was measured in only two cultivars: Mars and Lemont. Three different fields of each cultivar were monitored, designated as north (N), center (C), and south (S). The results from this season were not as uniform as in 1993 (see Figure 5.4). The average daily Eh of the three Lemont fields was more positive than the average daily Eh of the three Mars fields (see Figure 5.4d). However, this trend is not evident when the individual fields of the two varieties were compared. Though the Lemont center and south fields were more positive than the Mars center and south fields (p<0.01), the Eh values in the north field of both cultivars were not significantly different (see figures 5.4a-c).

Similarity of the Eh in only the north field can be attributed to significant differences found between the seasonal Eh pattern of the three Lemont fields. A two-way ANOVA test and subsequent post-hoc tests showed that the north Lemont field had a significantly lower daily seasonal Eh pattern than both the center and the south Lemont fields (p<0.05). However, the center and south fields were not different from each other. The daily seasonal Eh patterns for the three Mars fields were not found to be significantly different. When comparing the Eh trends of the two cultivars, it is necessary to realize that the three Lemont fields were not successful replicates. It is possible that some other variable interfered with the measurement during that season.

In 1993, the ferrous ion concentration was also measured in ten different cultivars. Cultivars in field positions 1-6 (LeBonnet, Lemont,
Figure 5.4. Eh of Mars and Lemont fields in China, 1995. Shown are (a) north field, (b) center field, (c) south field, and (d) average of three fields. In (d) the curve fit equations and r² values follow: 
y = -247.798LOG(x) + 227.420, r² = 0.780, and y = -214.781LOG(x) + 296.407, r² = 0.683 for Mars and Lemont, respectively.
Dawn, Katy, Della, IR-36) and cultivars in field positions 7-10 (Mars, Brazos, Labelle, and Jasmine) were sampled on alternating weeks. Therefore, statistical tests could only be used within the two groups of varieties. There were no significant differences within the groups in either the top or bottom 5 cm of soil. In order to compare general trends, the average ferrous ion concentration of both groups were plotted against the same time axis (see Figure 5.5a-b). No differences are evident between the two groups of cultivars in either depth.

The average ferrous ion concentration for both cultivar groups tends to increase in both the top and bottom 5 cm of soil after flooding. Both depths showed similar responses with time, however, the bottom depth was consistently lower than the top depth. In addition, the top had a sharper slope till day 30, and then experienced a leveling off till the end of the season. The bottom depth experienced a more linear increase throughout the entire season.

Since no differences were observed between the cultivar groups, the data from the two groups were combined and fit with a logarithmic curve fit (r^2=0.944 and 0.839 for the top and bottom, respectively) (see Figure 5.5c). The lower r^2 for the bottom depth is due to the greater linearity of the ferrous ion concentration in the bottom 5 cm (linear regressions were 0.844 and 0.961 for the top and bottom, respectively).

The pH was also monitored in the ten cultivar fields in 1993. The same cultivar groups were sampled as for the ferrous ion concentration. The pH values for each cultivar group for both five cm depths were plotted together against time (see Figure 5.6). The pH of the soil was initially acidic, with values lower than 5.5. In the top depth, a maximum was reached near day 30 in all fields, at a value of 5.93 and 5.985 for fields 1-6.
Figure 5.5. The average ferrous ion concentration vs. time for (a) both groups of cultivars in the 0-5 cm and 5-10 cm soil increments and (b) both depths for all cultivars fitted with a logarithmic curve fit. Error bars are standard deviations.
Figure 5.6. The average pH within each cultivar group vs. time for (a) 0.5 cm and (b) 5-10 cm. Error bars are standard deviations.
and 7-10, respectively (see Figure 5.6a). After day 30, pH values in this depth tended to level off until the end of the season. The bottom depth experienced a similar increase to 30 DAF, followed by a slight gradual increase to a maximum late in the season, 6.07 (63 DAF) and 6.23 (51 DAF) for fields 1-6 and 7-10, respectively (see Figure 5.6b).

In order to compare the seasonal trends among all cultivars, pH values were averaged over all sampling dates after day 30, which marked the end of the sharp increase early in the season. When these averaged values were compared between depths, the top depth was consistently lower than the bottom depth (see Figure 5.7). The top 5 cm for all cultivars had a grand mean of 5.717, and the bottom 5 cm had a grand mean of 5.99.

The average values of pH after day 30 were compared among cultivars for both depths. The top depth showed more differences than did the bottom depth. In the top depth, there were two groups significantly different from one another, cultivars Della (5), LeBonnet (1), and IR 36 (6) and cultivars Brazos (8), Labelle (9), and Jasmine (10). The latter group of cultivars had a higher average pH value than the prior. Each cultivar in these groups was found to be significantly different from at least one other cultivar in the other group. Table 5.2 lists the specific differences of these groups. In the bottom depth, only two cultivars were

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>1 (Lebonnet)</th>
<th>5 (Della)</th>
<th>6 (IR-36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (Brazos)</td>
<td>0.065</td>
<td>0.002**</td>
<td>0.172</td>
</tr>
<tr>
<td>9 (Labelle)</td>
<td>0.003**</td>
<td>0.000**</td>
<td>0.012*</td>
</tr>
<tr>
<td>10 (Jasmine)</td>
<td>0.049*</td>
<td>0.001**</td>
<td>0.142</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01.
Figure 5.7. The average pH after 30 DAF plotted against cultivar field position and for both 5 cm soil depths. Cultivars begin with 1-LeBonnet and end with 10-Jasmine. Also shown are the grand means for each depth.
different, Della (5) and Brazos (8). The average pH of Brazos was higher than the average pH of Della.

Total organic carbon measurements (TOC) were made throughout the 1993 season for the same sampling groups as the ferrous ion concentration and pH determinations. There were no significant differences over time in any field or at either depth. Therefore, the averaged TOC values were compared among cultivars, including the non-vegetated control field. Results from a post-hoc test revealed that significant differences occurred mostly in the top depth (see Table 5.3). Only one difference is evident in the bottom 5 cm, between cultivars in position 6 and 3 (p=0.048).

Table 5.3. Pairwise comparison probabilities for TOC differences among cultivars in the top 0-5 cm depth.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.008**</td>
<td>0.000**</td>
<td>0.000**</td>
</tr>
<tr>
<td>8</td>
<td>0.496</td>
<td>0.009**</td>
<td>0.004**</td>
</tr>
<tr>
<td>10</td>
<td>0.867</td>
<td>0.061</td>
<td>0.034*</td>
</tr>
<tr>
<td>11</td>
<td>0.433</td>
<td>0.006**</td>
<td>0.003**</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01.

Unlike the sporadic differences over time and between cultivars, TOC varied consistently between the two depths. In order to display these depth differences more clearly, TOC values for each cultivar were averaged over the whole season and plotted against cultivar position, including the non-vegetated or control field (see Figure 5.8). Except for field 7 (Mars), the top depth had a higher percentage of organic carbon than the bottom depth. The top 5 cm grand mean was 1.34 and the bottom average was 1.23.
Figure 5.8. Seasonal average percent total organic carbon vs. field position for both 5 cm soil depths. Also shown are the grand means for each depth.
Finally, methane production rates were measured in two cultivars, Mars and Lemont, in China, Texas, in 1995. Samples were gathered three times during the season, on days 21, 41, and 62 DAF, and from the north, center, and south fields. Results indicate that methane production rates increase throughout the season for both cultivars (see Figure 5.9). The Lemont production rates have been described previously (see Chapter 3). Compared to other seasons, there was a lag in the increase of production rates in all three Lemont fields.

Statistical tests performed on the methane production data for the two cultivars (two-way anova - timexfield). Days 21 and 62 were found to be significantly different (p<0.01). All three Lemont fields were found to be significantly different from Mars north (p<0.05). Lemont center was not found to be significantly different than Mars center, however, the probability value was not much greater than 0.05 (p=0.082). Finally, Lemont south was significantly different than Mars south (p<0.05).
Figure 5.9. The methane production rates of the 1995 season vs. time, including (a) Lemont and (b) Mars north, center and south fields.
6. RESULTS OF THE IMPACT OF SOIL TEXTURE

Methane emission rates have been found to be higher in fields with higher sand percentage (Sass et al., 1994; Wang et al., 1990). The mechanism which produces this relationship is unclear. Studies were performed in the 1994-95 seasons to determine whether redox parameters varied in fields of different soil texture. This was accomplished by measuring the Eh, pH, and ferrous ion concentration throughout the two seasons. In addition, to ascertain whether substrate availability differed among the fields, algal mat development on the soil surface and root distribution were monitored in 1994. Finally, the most probable number, or MPN, tests were done on soils from both seasons to estimate the methanogenic bacteria population size in the different fields.

6.1. Soil Characteristics

Experiments were carried out over two years (1994-95) in the same fields in China, Texas, designated as the north, center, and south fields. Soil texture analyses were performed using soils taken from the fields each season. In 1994, soils were taken prior to flooding approximately every 100 m in a north-south gradient. In 1995, soils were taken 17 DAF in the south end of each of the three fields.

Soil texture test results show that though there were differences in the relative values of the various fractions, the clay content decreased and the sand content increased from the north to south fields in both years (see Figure 6.1). A two-way ANOVA (field x year) revealed that the soil texture results were significantly different between the two years. Significant
Figure 6.1. The percent clay, sand, and silt of the (a) 1994 and (b) 1995 China, Texas north, center, and south fields. Error bars are standard deviations.
interactions were found between time and field for both percent sand and silt.

Since the years were significantly different and significant interactions were detected, one-way ANOVA tests were done on each year individually to determine if a change in the method between the years affected the results. In both years, all three fields had different clay content (N<C<S), and the north field was significantly higher than both the center and south fields in sand content. There were no differences in silt percentage in 1994, however, in 1995 all three fields were different from each other.

Since the same trends among the fields are evident in both years, it appears that differences between the years were not due to changes in the testing, but to the heterogeneity of the soil in the fields. The areas sampled, as well as the times of sampling, may have contributed to the measured differences.

Sass et al. (1994) demonstrated that the metal concentration in soils increases with increasing clay content. Metal analysis of the 1994 soils support this (see Table 6.1). The concentration of the metals increase from the north to the south fields.
Table 6.1. The concentration of metals in the north, center, and south fields in 1994. The units of the metal concentration are in μg/g. Errors are standard deviations.

<table>
<thead>
<tr>
<th>Metal/Field</th>
<th>North</th>
<th>Center</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>9236 (3890)</td>
<td>14889 (3477)</td>
<td>19317 (3598)</td>
</tr>
<tr>
<td>Arsenic</td>
<td>18 (5.3)</td>
<td>25 (4.6)</td>
<td>35 (9.2)</td>
</tr>
<tr>
<td>Barium</td>
<td>84 (4.7)</td>
<td>99 (3.6)</td>
<td>119 (5.3)</td>
</tr>
<tr>
<td>Beryllium</td>
<td>0.44 (-)</td>
<td>0.44 (0.07)</td>
<td>0.71 (0.09)</td>
</tr>
<tr>
<td>Calcium</td>
<td>2359 (101)</td>
<td>2890 (78)</td>
<td>3713 (178)</td>
</tr>
<tr>
<td>Cobalt</td>
<td>1.82 (0.29)</td>
<td>2.61 (0.3)</td>
<td>2.72 (0.26)</td>
</tr>
<tr>
<td>Chromium</td>
<td>7.5 (2.8)</td>
<td>11.5 (2.2)</td>
<td>14.2 (3.1)</td>
</tr>
<tr>
<td>Copper</td>
<td>4.5 (0.62)</td>
<td>5.4 (0.45)</td>
<td>6.59 (0.60)</td>
</tr>
<tr>
<td>Iron</td>
<td>6570 (1449)</td>
<td>9415 (909)</td>
<td>11348 (1155)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>905 (239)</td>
<td>1347 (185)</td>
<td>1697 (238)</td>
</tr>
<tr>
<td>Manganese</td>
<td>44 (4)</td>
<td>66 (6)</td>
<td>71 (4)</td>
</tr>
<tr>
<td>Sodium</td>
<td>150 (14)</td>
<td>145 (13)</td>
<td>160 (12)</td>
</tr>
<tr>
<td>Nickel</td>
<td>3.31 (1.01)</td>
<td>5.28 (0.75)</td>
<td>6.47 (1.07)</td>
</tr>
<tr>
<td>Strontium</td>
<td>18.1 (0.88)</td>
<td>23.2 (1.32)</td>
<td>29.5 (1.62)</td>
</tr>
<tr>
<td>Vanadium</td>
<td>19.3 (4.09)</td>
<td>26.22 (3.13)</td>
<td>31.07 (4.27)</td>
</tr>
<tr>
<td>Zinc</td>
<td>27.31 (10.49)</td>
<td>45.06 (20.09)</td>
<td>42.19 (13.82)</td>
</tr>
</tbody>
</table>

6.2. Redox Characteristics

Redox parameters were measured in these fields to observe if soil redox conditions vary in fields of varying soil texture. The Eh, ferrous ion concentration, and pH were measured in the 1994 season (see Figure 6.2). In these figures, the data shown represent averages over all four 2.5 cm depths. The overall trends of each of these variables did not show any variation for the three fields. Two-way ANOVA tests (field x year) showed significant differences for all three variables over time, but no significant differences among the fields (p<0.01). These tests were performed using the mean of each sampling date after 21 DAF. No dates before 21 DAF
Figure 6.2. 1994 data vs. time for (a) Eh, (b) ferrous ion concentration, and (c) pH. Data for north center and south fields are shown.
were included since the sampling in the center field did not begin until that time.

In 1995, the Eh was measured in each of the three fields (see Figure 6.3). The Eh data from Lemont lack any uniformity among the north, center, and south fields (see Figure 6.3a). Statistical tests showed that the north field was significantly lower than the other two fields, however, the center and south fields were not different from each other. The Eh data from Mars were similar among the three fields with no significant differences found (see Figure 6.3b).

6.3. Methane Production Rates

The methane production rates were also measured in the two seasons in order to determine if soils with different clay percentages produce a different amount of methane. Results from both years indicate that the north field does produce a greater amount of methane than either the center or the south fields (see Figure 6.4). The relationship between the center and south fields are not as clear. In 1994, the two fields appear to be producing methane at similar rates by 64 DAF (see Figure 6.4a). In 1995, however, the center field was outproducing the south field by 62 DAF.

The relative amounts of methane produced in the two years were also quite different. By 64 DAF, there was five times more methane produced in 1994 than in 1995 for the north and center fields. Even more different, the south field was producing greater than 1000 mg CH4 m\(^{-2}\) d\(^{-1}\) in 1994 was producing negligible amounts in 1995.
Figure 6.3. The Eh vs. time from the 1995 season for (a) Lemont and (b) Mars showing the north, center, and south field seasonal trends.
Figure 6.4. The methane production rate vs. time for the (a) 1994 and (b) 1995 seasons showing results from the north, center, and south fields.
6.4. Algal Mat, Root Distribution, and MPN Analyses

Devices were buried near 30 DAF and removed at the end of the season to observe growth of the algal mat. There was no apparent development of an algal mat in the north, center, or south fields in either Lemont or the non-rice area.

Root distribution was measured in soil cores taken on 14, 29, 42, 57, and 73 DAF in the north and south fields only. Statistical tests indicate no significant differences over time or between fields, however, there were differences over the four depths (see Figure 6.5). In the north field, the top depth had significantly more root mass than the bottom two depths, 5-10 cm total, especially late in the season after 42 DAF. In the south field, the root mass in the top depth was significantly higher that all three of the other depths, 2.5-10 cm.

Most probable number experiments were performed on soils taken from all three Lemont fields in 1994 and 1995. In 1994, the soils were taken at the end of the season near harvesting. They were taken directly from the methane flux frames located in each field. In 1995, the soils were taken 17 DAF, in the south end of each field.

In 1994, the MPN of methanogenic bacteria was similar in the north and center fields, with the south field having values one order of magnitude lower (see Figure 6.6). In 1995, all the fields had values similar to the south field in 1994. The population estimates in 1994 are 2975, 2705, and 118 for the north, center, and south fields, respectively. In 1995, the estimates were 192, 502, and 252 for the same fields.
Figure 6.5. The mean weight of roots in the north and south fields in (a) 0-2.5 cm, (b) 2.5-5 cm, (c) 5-7.5 cm, and (d) 7.5-10 cm depth increments. Error bars are standard deviations.
Figure 6.6. Most probable number results for the north, center, and south fields for both 1994 and 1995. Error bars are standard deviations.
7. DISCUSSION

Through the measurement of redox parameters and the methane production rate in fields, it is possible to more accurately describe reduction processes in rice paddy soils. The first section explains the inter-relationships between Eh, methane emission, methane production, ferrous ion concentration, and pH in rice soils. Special consideration will be given to processes that occur immediately after flooding. The second section will examine the effects of field drainage. The third and fourth sections study the use of different rice cultivars and the impact of soil texture on redox processes, respectively.

7.1. Seasonal Patterns

The seasonal trends for the redox potential, ferrous ion concentration, pH, and methane production have been shown to be similar to other studies. Measured together for the first time in Texas rice paddies, it is possible to combine the different results for a complete interpretation of the redox processes in the soil. Although it is impossible to discern activities of a particular microbe or microbial community, one can learn about the general state of the soil, and the switching from aerobic to facultative anaerobic to obligate anaerobic conditions. Also, the differences among the various depths measured will be investigated in light of the overall redox processes occurring in the soil.

The redox potential is a useful tool in estimating the reducing power of the soil. Laboratory experiments suggest that methane production begins at values between -150 and -160 mV, with a negative exponential
relationship between methane production and Eh between -150 and -230 mV (Wang et al., 1993). Mayer and Conrad (1990) show methane production initiating at -100 mV in laboratory incubations. Table 7.1 shows the day which methane emissions were first detected in field and outdoor pot studies in Texas and the corresponding Eh values. Methane emission rates were used to estimate the initiation of methane generation in the soil since experimental evidence has shown the similarity between the start-dates of methane emission and production rates.

Table 7.1. The date of initial measured methane emissions (given in DAF) and the corresponding redox potential (in mV) for 6 different fields over two seasons. Also included are the average start date and Eh with standard deviations given in parenthesis.

<table>
<thead>
<tr>
<th>Year, Field Site</th>
<th>CH4 Emission Start Date (DAF)</th>
<th>Eh (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994 Field North</td>
<td>6</td>
<td>210</td>
</tr>
<tr>
<td>1994 Field South</td>
<td>14</td>
<td>70.5</td>
</tr>
<tr>
<td>1994 Outdoor Pot</td>
<td>16</td>
<td>57</td>
</tr>
<tr>
<td>1995 Field North</td>
<td>14</td>
<td>107</td>
</tr>
<tr>
<td>1995 Field Center</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>1995 Field South</td>
<td>16</td>
<td>74</td>
</tr>
<tr>
<td>Average (SD)</td>
<td>14 (3.9)</td>
<td>93 (61)</td>
</tr>
</tbody>
</table>

The average measured start day for methane emissions was 14 DAF. The initial start day was named as the first day of positive methane flux measurements for each season. The average Eh value for this day was 93 mV. This value is more positive than those reported by Wang et al. (1993b) and Mayer and Conrad (1990).

This may be due to just one or several factors. First, both of the studies reported were based on experiments performed in a laboratory. The soils used in the experiments were homogenized unlike the very
heterogeneous mixture of in-situ paddy soils. Field studies in Japan in 1989 indicate similar patterns to those reported in this study (Minami, 1990). Positive flux measurements there were first measured close to 20 DAF, and the Eh values were all well above 0 mV, some reaching to above +400 mV (Minami, 1990). Eh values of -150 mV at 5 cm beneath the soil water interface occurred near 40 DAF.

The heterogeneous nature of the soil may facilitate the formation of micro environments where methane-producing conditions are present, which may not be able to be measured by the platinum electrodes used for monitoring Eh. The platinum tips are approximately 7 mm long with a diameter of 1 mm, and thus only measure a small portion of the soil. When several electrodes are used, their combined value is an average representation of the soil Eh.

Once the soil becomes uniformly reduced, methane emission rates increase sharply. This uniform reduction is evident in the lower Eh values and the decrease in variation among Eh readings. A negative plateau in Eh data in our fields was reached near 20 DAF and corresponds not to the initial methane emission, but to the sharp increase in emission rates characteristic to emission patterns previously reported (Sass et al. 1992, 1991a, 1991b, 1990, Schütz et al., 1989). Figure 7.1 shows the Eh and methane flux data for the first 30 days after flooding for Lemont north (1994) and Lemont in the outdoor pot study. It appears that methane emissions increase quickly once Eh values reach a minimum near or below -100 mV close to 23 DAF.

Also, there is a corresponding trend in the variance associated with the measurement of Eh. The standard deviation of the redox potential measurements tends to decrease through the season. Figure 7.2 displays the
Figure 7.1. Methane emission rates and $\text{Eh}$ plotted against time for (a) Lemont Central, China, Texas, 1994, and (b) Lemont, outdoor potted rice study, 1994.
Figure 7.2. The standard deviation of the redox potential and methane emission rate plotted against time for (a) Lemont Central, China, Texas, 1994, and (b) Lemont Control, Rosenberg, Texas, 1995.
standard deviation of two depths measured during consecutive seasons in different locations: 1994 China, Texas Lemont center field, and 1995 Rosenberg Lemont control field. Data from the depths 2.5-5 and 7.5-10 cm are shown, along with methane emission rates, plotted against time. These results show a decline in the standard deviation of Eh readings to minimum values near 27 DAF. The bottom depth in the Rosenberg field (Figure 7.2b) shows a less dramatic decline in error throughout the season which may be due to partial drains occurring early in the season. The overall decline in error coincides with the drop in Eh that occurred on 23 DAF and the subsequent increase in methane emissions. As the soil becomes uniformly reduced, it is logical that Eh readings would have less variation.

Once low Eh values are established, values range between -100 and -200 mV. This matches previous results which show methane production to begin between -100 and -150 mV (Wang et al., 1993; Mayer and Conrad, 1990). Results from Texas rice paddies indicate an exponential increase in methane during the time after Eh values reach those levels. Eh data from Rosenberg (1995) plotted against the natural log of methane emission rates led to a significant correlation for both the 2.5-5 cm and 7.5-10 cm regions $r^2=0.900$ and 0.749, respectively (N=9). Additionally, data from the outdoor pot study plotted similarly were also significant. The five control pots had an $r^2$ of 0.925, and the five drain pots averaged an $r^2$ of 0.692 (N=9).

Methane production is the rate at which methane is produced in the soil, and methane flux is the rate at which the gas is emitted. Both appear to start at the same time after flooding. Early in the season, production values were similar to and even lower than emission rates during the 1994
and 1995 China, Texas seasons. Having production rates lower than emission rates is most likely due to the heterogeneity of the soil. As shown previously, before 30 DAF, the soil is not uniformly reduced as evidenced by Eh and emission rate data. Methane production cores are 2.5 cm in diameter and may not have been in the region of maximum production early in the season. Sass et al. (1990) showed that cores taken in the rice row had higher production rates than those taken one quarter or one half of the row from the plant early in the season, near 30 DAF. Measurements in these studies were taken one quarter of the row from the rice plant.

Results from Sass et al. (1990) show that by 45 DAF rates were highest in the two positions away from the rice row. Similarly, by 41 DAF in 1994, production rates one quarter of the way from the rice plant were higher than emission rates, with a 31% oxidation rate estimation. Alternatively, in 1995, measurements on 41 DAF showed emission rates were still higher than production rates. The 1995 season was marked by higher redox values and lower methane production and emission rates. In that season, day 41 results matched those of a normal early season (before day 30). The differences between 1994 and 1995 will be covered in the Chapter on soil texture.

By 62 DAF, both years had significantly larger differences between production and flux. Emission rates were 440.35 and 58.87 mg CH4 m^-2 d^-1 in 1994 and 1995, respectively. These correspond to oxidation estimates of 57% and 73% for the two years. Overall, production/emission ratios increase over the season. This may be due to greater oxidation occurring in the soil or at the soil-water interface or to the method used for determining the production of methane. By incubating soil cores under
ideal anaerobic conditions, one may stimulate methane production, especially with substrates from roots cut from the coring process.

Methane emission and production rates displayed similar increases with respect to redox potentials. The ferrous ion concentration also showed increases, however, they were noticeably earlier in the season (see Figure 7.3). In Figure 7.3a, 1994 data for methane emission, production and ferrous ion concentration (of depth 2.5-5 cm) are plotted on the same axis opposite of the Eh (of depth 2.5-5 cm). As described, the Eh drops after flooding. Where the emission and production begin increasing near 20 DAF when the Eh is -100 mV, the ferrous ion concentration climbs immediately as the Eh decreases. Patrick and Henderson (1981) state that the critical Eh for iron reduction is between 0 and +100 mV for a two week laboratory incubation. Ferrous ion concentration increase in this study appears to occur at potentials higher than 200 mV. However, this may be due to reduction occurring in microzones which have lower potentials.

The ferrous ion concentration stabilizes near 25 DAF, which is also marked by the stabilization of Eh measurements near -150 mV and the sharp increase in methane emission rates. It appears that the reduction of iron plays a significant role in determining the redox state of the soil. The iron system has been described as the most important redox buffer system in rice soils (Neue and Sass, 1994). Neue and Sass (1994) state that the rate of decrease in Eh in paddy soils is determined chiefly by the iron system, since the nitrates and manganese oxide compounds have a small buffering effect. Soils high in both iron and organic matter may fall quickly to -50 mV, and then may decline slowly over many weeks. In addition, soils which are buffered strongly by the iron system may have a reduced
Figure 7.3. Eh, ferrous ion concentration, methane production and methane emission plotted against time for (a) Lemont Central, China, Texas, 1994, and (b) Lemont, China, Texas, 1993. The methane production is simulated in (b) as the emissions doubled. Graph (a) is based on data from the 2.5-5 cm depth, and graph (b) is based on data from the 0-5 cm depth.
tendency toward methane production. In our fields, it appears that the buffering effect of iron caused a delay in methane production until after 25 DAF, at which time the ferrous ion concentration reached a maximum that was maintained for the remainder of the season.

Figure 7.3b shows the Eh, ferrous ion concentration, methane flux and methane flux times two (to represent methane production values) plotted against time for the 1993 season. Though the Eh decline was slower, the iron started to increase sooner than the methane. The problem with this year may stem from the method used to measure the Eh. The electrodes were buried inside pipes placed in the soil. The pipes may have hindered root growth around the electrode, and thereby hindered the establishment of reduced conditions due to a lack of organic substrates.

Another relationship to examine is the one between pH and the ferrous ion concentration. Figure 7.4 shows the values of these two parameters collected in 1994 plotted against time. The increase in pH was found to correlate with the increase in ferrous ions. The reduction of iron is thought to be the main mechanism for the increase in the pH of acid soils immediately after flooding (Ponnamperuma, 1981). Ponnamperuma (1981) shows that the reduction of each ferric hydroxide complexes consumes three hydrogen ions. After the reduction of available iron is complete, the pH is most affected by the accumulation of CO2 or organic acids. These compounds would cause a drop in pH values, which is evident in our studies (see Figure 7.4a-c). In addition, this drop corresponds directly with the stabilization of the ferrous ion concentration.

In Figure 7.4, the 1994 pH and ferrous ion concentration curves are plotted for all four depths. As the depth increases, both the stabilization of a maximum ferrous concentration and the decrease in pH becomes less
Figure 7.4. The ferrous ion concentration and pH of Leman Central, China, Texas, 1994, plotted against time for the following depths: (a) 0.25 cm, (b) 2.5 cm, (c) 5.5 cm, and (d) 7.5 cm.
obvious. The lack of any decrease in pH could be due to the continual reduction of ferric complexes at lower depths which consumes protons. This could balance the increase in CO2 and organic acids which effectively lower the pH in higher depths.

Alternatively, this phenomenon could be explained by a lack of CO2 and organic acids in lower depths. This hypothesis was proposed to explain similar results from a study performed in 1992 (Lewis, 1994). This is corroborated by evidence from the other parameters and may stem from less microbial activity occurring in the deeper portions of the soil. The reduced activity may be due to a lack of substrates, smaller microbial populations, or lower temperatures. Less activity at lower depths would explain a smaller concentration of CO2 and organic acids, as well as a slower rate of increase for the ferrous ion concentration.

Results from 1993 were similar to those from 1994 for the top five cm of soil only (see Figure 7.5). Due to a lower frequency of sampling dates and homogenization of soil over a larger depth (0-5 cm) the 1994 trend is somewhat muted. For the top five cm, the pH does not drop as sharply, instead it exhibits a general slow decline from 30 DAF to the end of the season. The ferrous ion concentration stabilizes after 30 days. The bottom five cm shows a gradual increase for both the pH and the ferrous ion concentration throughout the season. Perhaps the pH of the bottom depth did not drop because the ferrous concentration never stabilized. The bottom depth is generally known for its slower reduction.

In general, the top depths (0-5 cm) showed the quickest response to flooding in all of the parameters, and the lower depths (5-10 cm) showed a lag in their response. These may be attributed to the distribution of substrates, especially those derived from plant roots. The growth of rice
Figure 7.5. The ferrous ion concentration and pH of Lemont, China, Texas, 1993, plotted against time for the following depths: (a) 0-5 cm and (b) 5-10 cm.
roots predominates in the top 5 cm, especially in the second 2.5 cm until the adventitious roots develop at the soil surface in the middle of the season. Additionally, the second 2.5 cm hosted the highest methane production rates until 64 DAF, when the top 2.5 cm also produced at high levels.

The bottom-most depth did not show similar responses until 50 DAF for ferrous ion concentration and Eh. This is indicative also of a lack of substrates at that low depth until late in the season. At this point they could be derived from the expanding root growth or from diffusion of substrates downward either from the floodwater, the biomass of bacterial populations in higher depths, or from the roots exudates above (Personal communication, Dr. Frank Fisher).

The smaller amount of substrates at lower depths may lead to reduced microbial biomass with less respiration. This would in turn lead to lower concentrations of CO2 and organic acids, which may contribute to the stabilization of the higher pH values at these depths. Additionally, the depths closest to the surface may experience the greatest substrate in-put, with enhanced microbial respiration leading to large build-ups of CO2 and organic acids. This is evidenced by the largest decrease in pH occurring in the top 5 cm of more than 1 pH unit.

By measuring Eh, ferrous ion concentration, pH, and methane production throughout several seasons and field conditions, a greater understanding of soil reduction processes is gained. The reduced conditions in the soil that occur after flooding appear to be dominated by the iron system. The increase in ferrous ions corresponds to a drop in Eh. In addition, it causes an increase in pH. Once the ferrous ion concentration stabilizes, the Eh stabilizes between -100 and -200 mV and the pH drops
at that time, organics are utilized as electron acceptors with the production
of methane resulting.

The production and emission of methane are similar until after 50
DAF, at which time the production rate is at least two times the emission
rate. This time, 50 DAF, also signifies the full reduction of the soil, or
when the bottom-most depth measured had Eh and ferrous ion
concentrations similar to the higher depths. The depths closest to the
surface are affected most quickly by flooding, and respond immediately in
all parameters.

7.2. Field Drainage Effects

Field drainage is a strong candidate for mitigation of methane
emissions from rice paddies. Methane emission and production rates are
known to decrease during field drainage (Sass et al., 1992; Yagi et al.,
decreased by 50% with one field drainage and almost 90% when fields
underwent three drainages during one season.

The objectives of this study were two-fold. The first was to
determine whether the main mechanism by which field drainage occurs is
due to an increase in methane oxidation or to a decrease in methane
production. Both of these would result in the decreases in methane
emission rates observed in drained rice paddies (Sass et al., 1992; Yagi et

This decrease in methane emission rates is one benefit of field
drainage, however, it has been suggested that drainage may also have
negative effects (Neue, 1993). The second objective of this study was to
negative effects (Neue, 1993). The second objective of this study was to assess the magnitude of these negative effects in Texas rice paddies. These negative impacts include the possible release of nitrous oxide (N\textsubscript{2}O) emissions and reduced rice grain yield.

Nitrous oxide (N\textsubscript{2}O) is an important greenhouse gas, as well as a participant in stratospheric ozone chemistry. N\textsubscript{2}O is emitted by anaerobic soils that undergo re-oxidation (Massscheleyn, et al., 1993; Hanif et al., 1986). Any increase in nitrous oxide emissions would severely undermine the effectiveness of field drainage as an overall mitigation tool.

Another possible negative consequence of field drainage is reduced grain yield. Neue and Sass (1994) stated that the rice plant is most sensitive to water loss during the reproductive stage, as it results in a high amount of sterility. In addition, water loss in the vegetative stage can also affect the grain yield by decreasing plant height, tiller number, and leaf area. However, other studies in Asia have shown that periods of aeration at the end of tillering and just before heading can improve grain yields (Yoshida, 1981). Therefore, in any drainage study, it is necessary to analyze the effect on grain yield.

The first objective was accomplished through the measurement of methane production rates and several redox parameters throughout drainage events in three consecutive seasons. These studies show that the methane production decreases, the redox potential increases, and both the ferrous ion concentration and soil pH decreases during a drain event.

The shift from a negative Eh to positive Eh occurs as soon as one day after draining in the top 2.5 cm of the soil. Results from 1994 indicate that the Eh increase affects the methane production first and then the ferrous ion production. By three days after draining, the average percent
respectively. By five days after flooding, the values had dropped further to 2% and 46%. This is probably due to the lower Eh at which methane production is initiated (-150 mV) as compared to the Eh at which iron begins to be reduced (+100 mV) (Wang et al., 1993, Patrick and Henderson, 1981). As Eh increases with aeration in drained soils, the more anaerobic processes will be affected first.

These changes occur as oxygen-filled air reenters the soil during a drainage event. These studies indicate that the infiltration of air into 10 cm of soil is not a quick process, rather it occurs over a period of a few days (see Figure 4.4). Results from 1994 show that the Eh of the bottom 5-10 cm remains significantly lower (<0 mV) than the top 5 cm for as long a time as 3-4 days after draining (see Table 4.3).

This slow downward trend is also visible in the reduction in ferrous ion concentration (see Figure 4.5). The ferrous ion concentration in the first and second 2.5 cm increments drop more quickly than the bottom 5 cm by three days after draining. These three depth increments were significantly different from each other at that time. By five days after draining, the top 5 cm was still significantly lower in ferrous ion concentration than the bottom 5 cm.

The methane production rates decreased so dramatically in all depths by three days after draining that no differences between the depths were evident. This suggests that although the Eh was still below 0 mV in the bottom five cm three days after draining, conditions were not suitable for the production of methane. This may be due to the lack of suitable substrates at those depths or the Eh was not low enough to enable methane production.
It is debatable as to whether the reduction in methane production occurs from increased oxidation or a decrease in production during a drainage event. Evidence here supports the latter scenario. First, the production rates measured by anaerobic incubations showed a decrease throughout the drain. If the decrease in methane emitted during a drain was due to increased oxidation, then when soil is placed under nitrogen and incubated for 48 hours, there should be detectable amounts of methane produced. This was not the case in these studies.

More supporting evidence for it being a decrease in production comes from the Eh data. Many studies have demonstrated that methane production begins at Eh values near -150 mV (Wang et al., 1993; Masscheleyn et al., 1993). As stated previously, in all three studies, the Eh values became positive as early as one day after draining and remained positive until after re-flooding in the top depths. Between 3-5 days after drainage, the Eh in the bottom 5 cm has also increased, until the Eh of ten cm of soil was uniformly greater than 0 mV. Therefore, it appears that since the soil becomes uniformly oxidized during a drainage event, conditions are not suitable for the production of methane.

In addition, Masscheleyn et al. (1993) suggested that once methane is formed it is slow to oxidize at moderately reduced (+100 - +400 mV) and oxidized (+400 - +600 mV) conditions. This was based on laboratory incubation experiments which monitored the methane production and oxidation rates of soils at a range of Eh values. This would also support the theory that production decreases rather than oxidation increases during a drain.

Finally, the reduction in the ferrous ion concentration also signifies a change from anaerobic to aerobic microorganisms. Iron reducing bacteria
are facultative anaerobes, capable of functioning in either reduced or moderately oxidized conditions. Ferrous ion production and oxidation appears to be a dynamic equilibrium in the soil system. Once the conditions promoting production of ferrous ions is removed, then the equilibrium shifts toward the complete conversion to ferric iron.

Also, the pH during a drainage time tends to decrease which may be to be due to the release of hydrogen ions from the oxidation of ferrous ions to ferric hydroxide complexes. The return of the pH to pre-flood conditions also supports that the soils is oxidized.

Although it appears probable that there is decrease in methane production during a drainage event, it is difficult to assess in-situ whether this is due to the methanogenic bacteria becoming dormant or to a shut down of the bacteria producing methanogenic substrates, such as the fermentative or hydrolytic bacteria which also require reduced conditions. From the data presented here, it appears that both groups experience a reduction in activity since the Eh values in ten cm of soil are greater than +100 mV, with a maximum of +400 mV, 5 days after a drainage.

The second objective of this study was to determine in a preliminary fashion, the extent of the negative effects of field drainage in our fields, specifically the emission of nitrous oxide gas and the reduction of grain yield. The main mechanism of nitrous oxide emission in reduced paddy soils (Eh < 200 mV) is thought to be through the denitrification pathway as follows:

\[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 \]
At Eh values between 0 and -200 mV, dinitrogen gas is the main end product, however, at Eh values between +400 and -100 mV, nitrous oxide is thought to be the main product formed (Masscheley et al., 1993). During field drainages, the Eh increases into the range at which nitrous oxide is the main product of denitrification.

Studies have shown that nitrogen oxide gas (NO) is a viable intermediate in the denitrification pathway (Lindsay, 1979). The pe+pH redox value can be a useful indicator as to which species are stable in the soil. The pe is a useful term that represents the negative log of the electron activity. It is calculated using the following formula:

\[ \text{pe} = \frac{\text{Eh}}{59.2 \text{ mV}} \quad @25 \, ^\circ\text{C} \]

The pe+pH values can range from 0, which represents the reduction of hydrogen, to 20.78, which represents the reduction of oxygen.

Theoretical calculations predict NO to be stable in the pe+pH range of 13-15. Hanif et al. (1986) showed that for basic soils (pH=8.3 prior to flooding), the pe+pH values throughout flooding and drying cycles were in the range of 13-15. This supports the theory that greater nitrogen loss does occur in fields that are alternatively wetted and dried compared to continually flooded fields.

The pe+pH values were calculated for the three fields used in the 1994 study. Results indicate that the pe+pH range for the 1994 fields are similar and decrease in all three fields until the drain event (see Figure 7.6a). Drains caused an increase in the redox value, to a maximum near 8. Redox values were also calculated for the 1995 Rosenberg fields (see Figure 7.6b), which have a similar pattern to the 1994 season. The
Figure 7.6. The average redox (pe+pH) vs. time for (a) the 1994 China, Texas season and (b) the 1995 Rosenberg, Texas season.
maximum value during the drainage event was also near 8. The highest 
pe+pH value was not during the drainage event, instead the maximum 
occurred near the beginning of the season, near a value of 10. These data 
 imply that the drained fields were not experiencing conditions suitable for 
the emission of N2O.

It appears that loss of nitrogen from drained fields is a greater threat 
in fields with alkaline soils. It may be possible to develop a positive 
relationship between soil alkalinity and nitrous oxide emissions during 
drainage events. Since these theoretical calculations can only be used to 
 imply in-situ conditions, experimental results are needed to support their 
conclusions.

The grain yield was not found to vary significantly between the three 
fields used in the 1994 China, Texas study. The grain yields for the north, 
center, and south fields were 5348, 5713, and 5719 kg ha\(^{-1}\), respectively. 
The highest grain yield was in a field that experienced two drainages. It 
appears that field drainage did not reduce grain yield.

In conclusion, these results suggest that field drainage causes a 
decrease in methane production due to the return of the soil to aerobic 
conditions. In addition, these studies resulted in no reduction in grain 
yield, and theoretical calculations predicted no increase in N2O emissions. 
However, several questions about the effectiveness of field drainage as a 
mitigation tool still remain.

The first question is the timing and duration of the drain. It appears 
that the timing of field drainage has been found to cause serious reductions 
in grain yield (Neue and Sass, 1994; DeDatta, 1981). Though it was not 
true in our case, further studies should be conducted to determine the ideal 
time for drainage to ensure maximum methane gas reduction with no cost
to grain yield. In addition, grain yield may be enhanced through field
drainage at specific times during the season (Yoshida, 1981).

The duration of field drainage also seems important. The first drain
in our 1994 study resulted in minimal reduction in methane production.
This may be due to the timing of the drain in the season, or to the fact that
heavy rains caused the drain to be cut short to only 3 days. It may be that
the soil needs a minimum of time after a positive Eh is reached before
anaerobic microbial systems are turned off and substrates are depleted.

In addition, it seems that the timing and duration of drains is crucial
for minimizing the cost of water management to the industry. It may be
possible to utilize evaporative water loss as an effective way to establish a
drain. However, preliminary studies in this area have inconclusive results
as to whether it lowers methane emissions (Personal communication, Dr.
Ron Sass). Further experiments are needed in this area.

The effectiveness of field drainage for the lowering of methane
emissions from rice fields cannot be denied. The main mechanism for this
appears to be the inhibition of the production of methane by the rising of
the Eh, thus indicating aerobic conditions. Many factors need to be
considered before it can truly be implemented into farming regime. The
negative effects of drainages (increased N2O emissions and reduced grain
yields) did not appear to be significant in our studies, although more direct
and detailed studies are needed for the N2O emissions. The next step
should also include the development of exact strategies involving duration
and timing of drain events to ensure maximum reduction in methane
emissions without causing loss of grain production.
Different rice cultivars were found to have different methane emission rates. Whether methane emission rates are enhanced by either greater methane production in the soil or higher transport rates of methane through the plant or reduced by greater oxidation in the root zone is an important question. The first objective of this study was to study the redox state in soils of different rice cultivars to determine if oxygen transport in some cultivars affects the average redox state of the soil.

The second objective considers differences between rice and non-rice plots. Previous studies seem to conflict as to whether non-rice plots have different methane emission rates than rice plots (Cicerone et al., 1992; Nouchi, 1992; Sass et al., 1990; Σχη τζ et al., 1989). Cicerone et al. (1992) found that vegetation did not play a large role in the amount of methane released. A more important factor was the addition of organic matter which stimulated methane emission in both rice and non-rice plots similarly.

Contrary to these results, other studies have shown that non-rice plots emit much less methane than rice plots (Nouchi, 1992; Σχη τζ et al., 1992; Sass et al., 1990). Sass et al. (1990) have shown that non-rice plots emitted negligible amounts of methane compared to rice plots. In a field study in 1989, the average daily emission rates in bare soils were 7.9 and 4.4% of rice plot emission rates for Lake Charles and Beaumont, Texas soils, respectively. In laboratory experiments, Nouchi (1992) found methane emission rates were 20 times higher in vegetated pots than in bare pots.
These lower methane emissions in non-rice areas can be attributed to at least two different explanations. The first is the lack of methanogenic substrates provided by the rice roots. Sass et al. (1990) reported that methane emission rates in the bare plots did not reach significant levels until late in the season. This was partially attributed to the spread of rice plant roots into the bare area.

The second explanation is that in the non-rice area, the rice aerenchyma, the normal conduit for methane's release to the atmosphere, is not present. Cicerone et al. (1992) observed that bare plots resulted in methane emission rates with higher levels of variability since bubbling was the dominant release mechanism. In rice plots, the aerenchyma of the plants offered more continuous transport and less variability in measurement.

Experiments were performed in Texas rice paddies to determine if non-rice plots did emit different amounts of methane than rice plots. Also, if differences were detected, to ascertain whether it was due to the lack of reduction in the non-rice soil or to the lack of transport of the methane to the atmosphere. This was accomplished by measuring redox parameters in both rice and non-rice plots over three seasons in two field studies and one outdoor potted rice study.

Results from these seasons indicate large differences in methane emission and redox parameters between rice and non-rice plots. However, negligible differences were found among fields of different rice cultivars. Rice and non-rice plot differences will be discussed first.

Methane emission rates were different between the plots in all three seasons monitored (see Table 7.2). Also, there were significant differences
between the Eh, pH, and percent TOC for the two field types. However, no differences were found in the ferrous ion concentration.

Table 7.2. The average methane emission rates in rice and non-rice plots in the 1993-95 season. Units are mg CH4 m⁻² d⁻¹.

<table>
<thead>
<tr>
<th>Year</th>
<th>Rice (Lemont)</th>
<th>Non-rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>380.13</td>
<td>22.62</td>
</tr>
<tr>
<td>1994 (Potted rice)</td>
<td>103.40</td>
<td>12.05</td>
</tr>
<tr>
<td>1995</td>
<td>53.10</td>
<td>4.20</td>
</tr>
</tbody>
</table>

The seasonal Eh pattern of non-rice and rice plots varied among the years studied (see Figure 5.1). In 1993, no differences were visible between the two areas. However, in the 1994 outdoor pot and 1995 season, though the Eh dropped similarly in both fields, the non-rice area remained higher than the cultivar area from approximately 20 DAF throughout the remainder of the season.

The different trend in 1993 could be due to the method of measurement used that year. The cultivar redox potentials of this year were more positive than other years, as well (see section 3.1). PVC pipes were buried before flooding, and the electrodes were placed in the soil inside the pipes. Therefore the electrodes in the rice areas were shielded from the normal growth of roots. This shielding could have affected the Eh in two ways. First, rice roots are one possible source of substrates for microorganisms, and separation from them could result in a soil which is not as reduced (Schütz et al., 1989; Holzapfel-Pschorn and Seiler, 1986). The Eh of the field would then appear higher than normal. Second, rice roots transport oxygen into the reduced soil, and therefore the soil in the pipes could have been more reduced than the rest of the field. This would
lead to Eh values more negative than normal. The more positive Eh values measured in 1993 seems to suggest that the former scenario is occurring.

Changes in the methodology between 1993 and the other seasons resulted in much more reliable Eh data. Therefore, it is likely that the Eh data from the 1994 outdoor pot study and the 1995 field season are more reliable and suggest the true seasonal trends for rice and non-rice plots.

In both years (1994-95), redox values were similar in both plots until near 20 DAF. Then, the non-rice area remained high (close to 0 mV in 1994 and near +400 mV in 1995). As described previously, methane emission rates increase dramatically in rice fields near 23 DAF, which is also marked by a decrease of the Eh to values below -100 mV (see Chapter 3). Since the Eh leveled off at values above 0 mV before 23 DAF in the non-rice fields, it is likely that negligible amounts of methane were produced.

Though methane emission rates differed, the ferrous ion concentration trends were not different between the rice and non-rice plots. Iron reduction is mostly completed by 25 DAF (see Figure 5.2a), since it begins near +100 mV.

The absence of a further decrease in Eh past 20 DAF may be due to a lack of suitable substrates for the methanogenic bacteria. Schütz et al. (1989) suggested that large methane emission rates mid-season were due to root exudation by the rice plants. They found that the methane emission in rice plots decreased by approximately 25% during the second half of the vegetated period when either the aboveground biomass was reduced or the planting density was decreased. In addition, Sass et al. (1990) detected measurable amounts of methane in bare areas only late in the season, most probably due to the encroachment of rice roots from nearby rice plots.
Further evidence for there being lower methane production rates in non-rice areas is found in the pH and %TOC data. Significant differences were found in the top 5 cm of the soil between the rice and non-rice areas for these two parameters.

Early in the season, the pH increased similarly in both rice and non-rice fields, which is due primarily to the reduction of iron complexes to soluble ferrous ions (see Chapter 3). After 35 DAF, however, as the pH in the rice field decreased steadily, the pH in the non-rice plot remained high (see Figure 5.2b). The usual decrease in pH after day 35 can be attributed to the accumulation of carbon dioxide and low molecular weight organic acids (Ponnampерuma, 1981), most likely resulting from fermentation reactions and the oxidation of methane. In the non-rice field, methane was being produced at much lower levels and the Eh may not have been lowered sufficiently to initiate the reactions of fermentative bacteria. Therefore, there may not have been a sufficient build-up of CO2 or organic acids to cause a decrease in pH.

Also, the percent of total organic carbon was significantly higher in the non-rice plot than in the rice plot. This may be due to the smaller amount of microbial activity in the non-rice plot and the lingering of high molecular weight non-volatile organic compounds.

These results imply that there are different microbial communities being activated in rice and non-rice areas. The iron reducers are working similarly in the two plots, however, either the methanogens or the other fermentative bacteria which produce methanogenic substrates do not appear to be functioning in the non-rice areas. This may be due to a lack of suitable substrates originating from the rice plants.
Though the redox conditions vary between rice and non-rice plots, results from this study suggest that soil redox is not significantly different in fields of different cultivars. First, the Eh values were not consistently different among the ten cultivars grown in 1993 (see Figure 5.3). In addition, results from the 1995 season were inconclusive in proving that the Eh measurements in the two cultivars were really different, due to the lack of successful replication in the Lemont fields. Also in the 1993 study, the ferrous ion concentrations were not significantly different among the cultivars.

The pH and percent TOC showed differences between cultivars in 1993. The pH values were compared by averaging the values after 30 DAF. Two groups were found different for the top 5 cm: cultivars (5, 1, and 6) and cultivars (8, 9, and 10). Only cultivars 5 and 8 were different in the pH of the bottom 5 cm of soil. The percent TOC was averaged over the season and compared. The top 5 cm of soil had two groups different from each other: cultivars (1, 2, and 3) and cultivars (6, 8, 10, and 11). Only cultivars in positions 3 and 6 were different in the bottom depth.

As described previously, methane emission rates differed among the cultivars (see Figure 7.7). The seasonal methane emission rates from highest to lowest by cultivar position follow: 5, 7, 10, 1, 2, 3, 4, 8, 6, 9, and finally 11 (non-rice). From these average emission rates, it is clear that there is no correlation between pH and TOC with methane emission rate. Instead, differences in pH and TOC appear to be due to the field position and soil differences between fields. Noticeable differences in the elevation of the fields were evident that year (Personal communication, Dr. Frank Fisher). The higher areas may have experienced more frequent
partial drains and resulted in lower average pH values and higher TOC concentrations due to the soil in those fields being less reduced.

Finally, whereas no significant differences in Eh, pH, ferrous ion concentration, and total organic carbon were related to the specific cultivar, methane production rates do appear to differ by cultivar. As with methane emission rates, the cultivar Lemont was found to have a lower production rate than Mars in the 1995 season for at least two of the three fields (north and south). The center field had a probability value near significance (p=0.082).

These results imply that once a minimum Eh is reached, methane production processes are initiated. Unlike in the non-rice plots, this occurs in all of the cultivar plots. However, once methane production processes are started, it appears that other factors are present which work to control the amount of methane produced. It also seems that these factors are related to the specific rice cultivar.

At least two factors could be used to explain differences. The first may be any differences in the aerenchyma systems of the cultivars. This may result in different amounts of methane transported from the soil, or in different amounts of oxygen transported to the soil. In addition, different oxygen concentrations in the rice root zones could be due to different populations of Beggiatoa, a bacteria living in a loosely mutualistic relationship with rice roots (Brock and Madigan, 1991).

From these studies, these options do not appear to be main factors controlling the methane differences among cultivars. First, the Eh was not found to be different among the cultivars. If larger amounts of oxygen-filled air were transported into or formed in the soil, one might see variation in the Eh in the soil. None was detected. However, these
Figure 7.7. Comparison of the average methane emission rate for the ten cultivars planted in 1993. Cultivar labels are as follows: 1-Lebonnet, 2-Lemont, 3-Dawn, 4-Katy, 5-Della, 6-IR36, 7- Mars, 8-Brazos, 9-Labelle, 10-Jasmine, and 11-Non-rice.
measurements were made in the root zone and not in the narrow 1-2 mm rhizosphere region surrounding the roots where the oxygen concentration would be highest. More in depth studies are needed to determine any Eh differences in the rhizosphere region.

Though the Eh did not appear different among the cultivars, the methane production rates of the cultivars were different, in proportion to methane emission rates. Therefore, it appears that it is in the actual production of methane and not its transport out of the soil that is varying between cultivars. This is most likely due to the supply of organic substrates by the plant. This factor seemed to be key in the lack of methane produced in non-rice areas, and it may also be key in regulating the amount of methane produced within each cultivar. Further studies are needed on the amount and type of substrates supplied by each cultivar. Root growth rates and distributions may also be important.

7.4. Soil Texture Effects

Many studies have shown that the soil texture, or the sand/clay/silt ratio, of rice paddy soil can have a significant affect on the amount methane that is emitted (Sass et al., 1994 Wang et al., 1990). Sass et al. (1994) showed results from four seasons which showed that soils with a higher sand percentage results in a higher methane emission rate. Methane emission rate could be predicted with the following linear equation: CH4 Emission (g m⁻²) = 10.40 + 0.718*(Percent sand) (r²=0.999).

In preliminary experiments in China, Texas, the ferrous ion concentration and pH of soils of different soil texture were studied to determine why methane emissions were greater, or more specifically,
whether the higher emission rate was due to a higher production rate in sandy soils or a higher oxidation rate in clayey soils. The soils were not found to have significantly different ferrous ion concentration or pH values, although methane emission rates were greater in the sandier soils (Lewis, 1994). From the iron and pH data, it was proposed that greater oxidation in clayey soils may be main reason since there was no evidence in reduction or in the methane production results that production was greater in sandy soils. However, the results were inconclusive due to the small number of methane production samples taken and to the lack of any Eh data, and therefore they demanded further studies.

Therefore, these experiments were repeated and expanded using the cultivar Lemont in the 1994-95 seasons to meet two objectives. The first was to verify that the redox conditions in fields with different soil texture do not vary. Eh, ferrous ion concentration, and pH were monitored. The second was to determine the possible reason for higher methane emission rates in sandier soils. In order to accomplish this, methane production rates, algal mat development, and root distribution were also monitored. Most probable number, or MPN, tests were also done on the soils to estimate the methanogenic bacteria population size.

Results from redox monitoring in the three fields appear to support previous findings. In 1994, there were no significant differences among the three fields in pH, Eh, or ferrous ion concentration. In 1995, the results were not as clear. The three fields were again monitored for the cultivar Lemont and resulted in the north field having a significantly lower seasonal Eh than the other two fields. The center and south fields did not show the same decrease in Eh as the north field, and did not reach consistent negative values until 30 DAF for the south field and never for
the center field. The Eh in the north field dropped below 0 mV before 15 DAF.

The Eh was also monitored in three Mars fields in 1995. These fields gave results similar to 1994; there were no significant differences among the fields. In addition, the Lemont north field in 1995 was not significantly different than the three Mars fields. It appears that the Eh is not different in fields of different soil texture normally, however, in 1995 other factors may have caused variation in the Eh in the Lemont center and south fields.

This difference between the two seasons was also evident in the methane production results. First, it is necessary to state that the methane production rates were significantly different among the fields in both years. The north field had consistently higher production rates than either of the other fields. The center and south production rates were similar in 1994, but not in 1995. In 1995, the center field outproduced the south field by the end of the season.

Though the relative production rates were similar between the two years, the actual values were very different. Production rates were larger in 1994. The 1995 production rates for the three sampling dates were 21% or less than the 1994 rates. The methane emission rates on those days are compared similarly and the 1995/1994 ratios were 10%, 8%, and 13% for days 21, 41, and 62, respectively.

Combined with the anomalies found in the Eh data for the 1995 season, it appears that other factors were contributing to the higher Eh and lower production rates found in the center and south fields. In addition, despite appropriately low Eh values, the production rates in Lemont north in 1995 were also low compared to 1994. The cause for this change
between the seasons is unknown. However, it is still possible to state that both years' results showed that sandier soil had larger methane production rates ($N>C>S$).

One possible explanation for the higher production rate in sandy soils is the greater availability of substrates in those fields. Substrates could be derived from root exudation (including a wide range of root products and sloughage), algal mat development, or from the floodwater. Experiments were carried out to determine whether algal mat development or the growth of roots was more extensive in the sandy soils.

Results indicate that in 1994, algal mat development on the soil surface was not a significant contributor of substrates, as negligible growth was measured. In addition, root distribution one quarter of the way from the rice rows showed similar growth in both the north and south fields. Therefore, it does not appear from this data that greater production in the north field is due to easier substrate availability.

Another explanation for differences among the fields comes from the actual population of methanogenic bacteria. MPN tests showed that the north and center field populations were significantly larger than the south field in 1994.

MPN results also can be used to explain differences between the two years. In 1995, the fields were all similar and low in methanogenic bacteria. They were an order of magnitude lower in the center and north fields than in 1995. It appears as if the fields in 1995 have a smaller methanogenic population than in 1994.

Previous studies have showed that dried and flooded soils have similar methanogenic counts, $10^4$-$10^5$ (Mayer and Conrad, 1990). This suggests that bacteria do not die, but remain dormant for periods in which
the soil conditions are not reduced enough for their survival. The studies here propose an alternative theory in that conditions may change from year to year to alter the size of the bacteria population.

External factors may have caused a change in the population size. Factors seemed to have altered the redox state of the soil and the methane production. Measurements of plant growth during the same two seasons have also shown reductions in the rice biomass from 1994 to 1995 (Personal communication, Huang Yao). In addition, grain yield in 1995 was 42%, 31%, and 49% of the 1994 yields for Lemont north, center, and south, respectively. Whether these external factors were due to climate changes or field management changes is unknown. However, as all aspects of the field condition appear to have been altered, it is improbable that the bacteria population size could also have changed.

However, there are other explanations for the MPN data, including that the test may have some intrinsic inaccuracies. The test is very difficult and may not be completely reliable. For example, the inoculation process was not done under completely anaerobic environment. In addition, the tubes were not shaken constantly during incubation and therefore the substrates and soil particles may not have been uniformly distributed throughout the medium.

Also, the acetate concentration of 80 mM may have been too high and had an inhibitory effect on the growth of methanogenic bacteria (Personal communication, Dr. Frank Fisher). This is based on laboratory soil slurry incubations which were spiked with different acetate concentrations. Concentrations higher than 80 mM were found to have reduced amounts of methane produced, however, this may have been due to
changes in the soil consistency. Additions of acetate tended to increase the viscosity of the soil slurries.

Finally, the amount of gas that was collectable in the tubes was the minimum amount needed for injection into the gas chromatograph. Only 4-5 mL was able to be withdrawn. Therefore, it was not possible to repeat measurements, and the small sample size could have resulted in incorrect data. It was very difficult to determine whether a sample was greater or less than a blank.

The results from these experiments support the theory that more methane is produced as well as emitted from sandier soils. It appears as with fields of different cultivars that fields of different soil texture do not have different redox conditions. Once a minimum Eh is reached, methane is produced. However, other factors can determine how much methane is produced. Whether this is due to larger methanogenic populations is not definite. Populations may be larger due to the larger pore spaces in sandy soils, which may aid in the distribution of substrates.

More studies are needed, including the measurement of methanogenic populations in soils of different texture. Also, tests here were only done for acetate utilizing bacteria. It would be beneficial to make estimates of the carbon dioxide reducing methanogens.

7.5. Conclusions

Methane emission from rice paddies is one of the largest anthropogenic sources of methane gas release. Reduction in the soil after flooding creates the perfect conditions for the production of methane gas. Studies here have shown that flooding immediately causes a lowering in the
redox potential, most likely due to the reduction of iron. As iron is reduced, the pH increases and the Eh drops further. When it reaches a minimum below -100 mV, the production of methane increases exponentially.

The power of the iron system to buffer the redox state of the soil is evident in the lack of methane formed until a ferrous ion concentration maximum is reached. In addition the pH decreases once the iron reduction reaction equilibrates, there is subsequent build-up of organic acids and carbon dioxide.

Methods to reduce methane emission from rice fields require the switch from anaerobic to aerobic conditions. This can most easily be accomplished through field drainage, which allows oxygen-filled air to reenter the soil. Studies here have found that this effectively stops methane production. The possible negative effects of increased N2O emissions or reduced grain yield were not evident. Future work should cover the duration and timing of drainages.

Another method to effectively mitigate methane emissions from rice fields involves the use of different rice cultivars. Cultivars emit different amounts of methane, however, the redox conditions in the soil are similar among varieties. This is unlike the non-rice plots which experience no further decrease in the redox potential once iron is reduced. It appears that once the anaerobic conditions are met, other factors work to control the amount of methane released from fields of different rice varieties. Work is needed to further investigate the root exudation of rice cultivars.

Soil texture is another factor that contributes to control methane emissions. Studies here have found that redox conditions do not vary in
fields of different soil texture, however, methanogenic bacteria population size may be an important determinant.

Overall, it appears that the reduced state of the soil is an accurate indicator for whether or not methane will be produced. However, it may not be as useful for determining the amount of methane that will be produced or emitted. The processes that work to control the production of methane are complicated and involve microbial, plant, and soil interactions held together by a web of biochemical and physical reactions. However, the measurement of the state of reduction of the soil, through the pH, Eh, and ferrous ion concentration, will remain a crucial indicator of the extent to which those reactions are occurring.
BIBLIOGRAPHY


Soil Redox and pH Effects on Methane Production in a Flooded Rice 

Whiting, Gary J. and Jeffrey P. Chanton. Primary Production Control of 


Yagi, K. and K. Minami. Effect of Organic Matter Application on 

and Thai Paddy Fields. In: *Proceedings of CH4 and N2O 
Workshop*. N.I.A.E.S. (National Institute of Agro-Environmental 
Sciences), Tsukuba, Japan (1992).

International Rice Research Institute), Los Baños, Philippines (1981).

Yu, T. *Physical Chemistry of Paddy Soils*. Springer-Verlag, New York 
(1985).