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PRODUCTION AND EMISSION OF METHANE FROM
EXPERIMENTAL PADDY SOILS

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Shan Ney Huang

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PRODUCTION AND EMISSION OF METHANE FROM EXPERIMENTAL PADDY SOILS

By

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ABSTRACT

PRODUCTION AND EMISSION OF METHANE FROM EXPERIMENTAL PADDY SOILS

By

Shan Ney Huang

Greater methane concentrations in the atmosphere has received increasing attention. Flooded rice culture is considered to be one of the major biogenic methane sources. Because of this, the effects of rice cultivation practices on the change of inorganics (NO_3^-, SO_4^{2-}) and organics (volatile fatty acids) were followed and the production and emission of methane from experimental soil studied under glasshouse conditions.

Initially, nitrate in pore water was high after the flooding of the dry soil, and then declined rapidly. Sulfate concentrations followed the same pattern; however, the rate of sulfate depletion was lower than that of nitrate. The concentration of volatile fatty acids increased gradually after dry soil flooding and reached a maximum concentration at 30 days after rice transplanting followed by a rapid decline.

Methane concentration in soils increased markedly during the first 20 days after flooding. The non-planted soil had higher methane concentration than the planted soil. Highest methane production rates were observed at 40 days after flooding, while the lowest rates of production were observed at 20 days. Rates of methane emission from planted soils ranged from 0.73 (0.13) to 15.69 (4.4) mg $CH_4/m^2/hr$. which was significantly (5%) higher than that observed from non-planted soils 0.11 (0.04) to 1.43 (0.6) mg $CH_4/m^2/hr$. The highest emission rates were observed during heading and flowering stage, while the lowest during the

tillering and maturing stage of growth. Most of the methane was emitted through the rice plant.

The addition of previous crop residues resulted in an increase in methane production and subsequent emission in both planted and non-planted treatments. However, no significant differences were observed in the production or emission of methane from fertilized treatments over the controls.

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INTRODUCTION

Importance of Methane Emission to the Atmosphere

Although methane (CH_4) is a relatively minor component of the global carbon cycle, its increased concentration in the atmosphere has recently received considerable attention (Cicerone and Shetter 1981, Seiler 1984) It is estimated that methane concentrations have increased 1.5 to 1.72% over the last 10 years (Seiler 1984, Ehhalt 1985). Because of the chemical and physicochemical properties of methane its increased concentration could greatly impact the chemistry of the atmosphere (Seiler 1984). Briefly:

(1) The increased concentrations of atmospheric methane would perturb the earth's radiation balance resulting in an increased "greenhouse effect" (Wang et al. 1976). Doubling the methane mixing ratio from the current concentrations of 1.6 ppmv would result in an increase in tropospheric temperature of 0.2 to 0.3°C.

(2) The increased oxidation of methane in the troposphere would result in a higher production of a series of gaseous intermediates and final products such as CO, H_2, HCHO, O_3 and other radicals (Levy 1971, McConnell et al. 1971, Wofsy 1972, Crutzen 1973). Changes in the concentration of these compounds will have a significant impact on the chemical composition of the troposphere.

(3) Methane is the predominant source of water vapor in the stratosphere since the products of its oxidation are CO_2 and water

vapor. Changes in the CH_4 mixing ratio will increase the flux of methane into the stratosphere and the H_2O mixing ratio. This will result in an impact on the distribution of OH and other odd hydrogen species in the stratosphere.

(4) The increase of tropospheric CH_4 will enhance the reaction between CH_4 and Cl (Stolarski and Cicerone 1974). The reaction $CH_4 + Cl \rightarrow HCl + CH_3$ is the dominant sink mechanism for the stratospheric chlorine radicals Cl and ClO. These compounds act as catalysts in the destructive reactions of stratospheric ozone.

Sources of atmospheric methane: From the ^{14}C content of atmospheric methane, Ehhalt (1974) and Ehhalt and Schmidt (1978) have estimated that about 80% of the methane is produced biogenically. The view that atmospheric methane is biogenic is strongly supported by Lovelock and Margulis (1974) who demonstrated that a purely abiogenic thermodynamic equilibrium would predict 29 orders of magnitude less CH_4 in the atmosphere than is currently reported. Ehhalt (1985) concluded that one major biogenic source of methane is wetlands (e.g. rice paddies) (Figure 1).

Methane is produced by a group of strict anaerobic bacteria in highly chemically reduced habitats (Smith and Hungate 1958) i.e. rumen, sludge digesters, swamps, and rice paddies. Methane produced in lake sediments must diffuse through an oxic-anoxic interface either at the sediment water interface or in the overlying water. Greater than 80 percent of the produced methane is oxidized to carbon dioxide at these interfaces (Rudd and Hamilton, 1979). However, methane produced in ruminants and rice paddies (Cicerone and Shetter 1981, Cicerone et al.

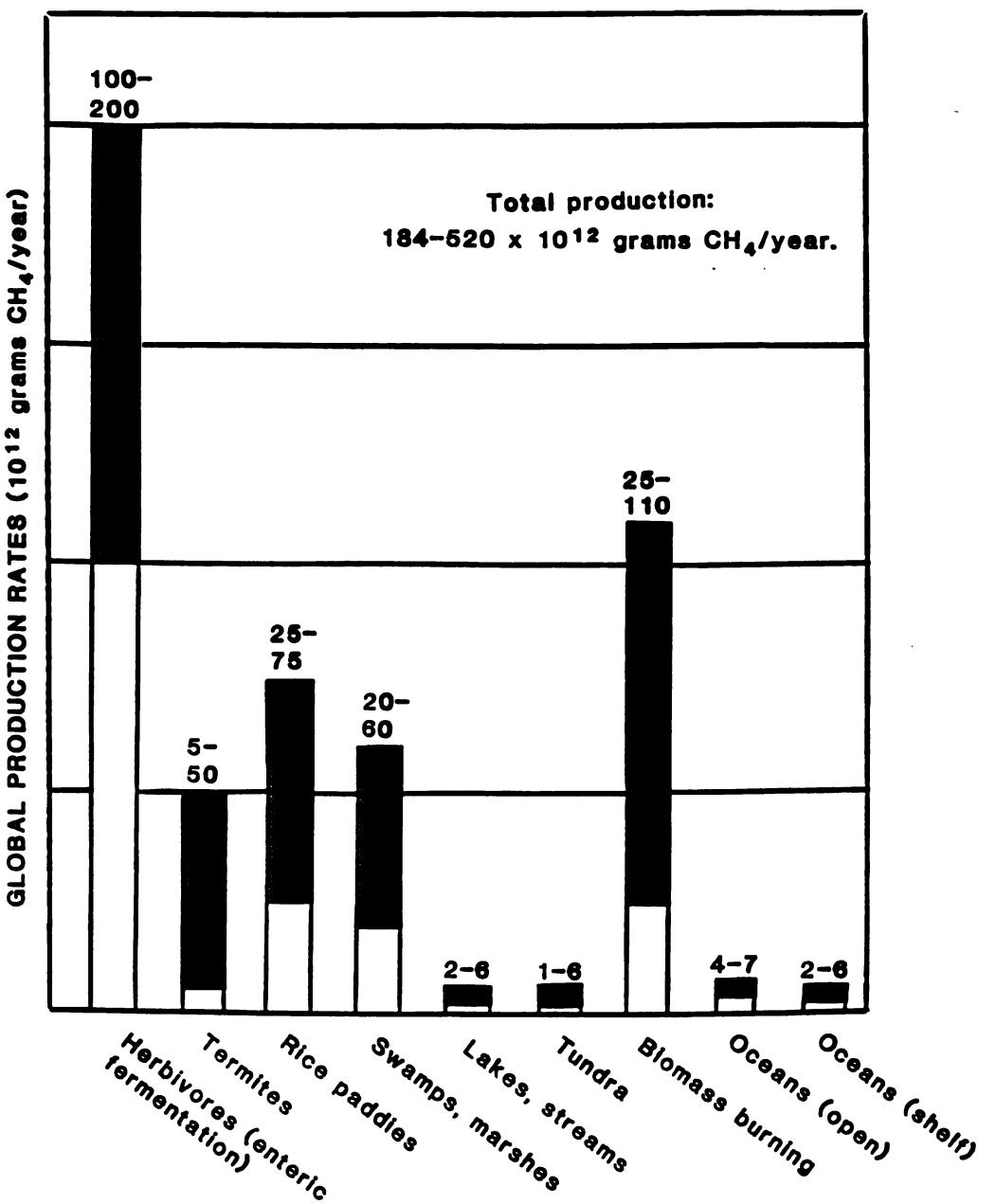

Figure 1. Sources of biogenic methane and production rates. (Ehhalt 1985).

1983) is emitted directly to the atmosphere and is only partially oxidized prior to reaching the atmosphere in rice paddies.

Official statistics (FAO Yearbook 1985) indicate that harvested rice paddies occupied 147 x 10^{10} m^2 in 1984. Therefore rice paddies are the largest wetland controlled by man. Since rice harvested acreage has increased only 2% annually since 1950, increased emissions of methane must be due to changes in the cultivars used or cultivation practices.

Importance of rice production: On a global basis rice ranks second to wheat in terms of the total harvested acreage. In terms of importance as a food crop, rice provides more calories per hectare than any other cereal crop (DeDatta 1981). At the average world yield, a hectare of rice could sustain 5.7 persons per year compared to 5.3 for maize and 4.1 for wheat (DeDatta 1981). It is estimated that 40% of the world population uses rice as a major source of calories. Rice provides more than half of the food for 1.3 billion people and 25-50% of the food for 400 million people. Rice is the only major food crop which grows primarily on the vast areas of flat, low-lying river basins and delta areas in Asia that are flooded to various depths during the monsoonal season. The significance of rice production is further emphasized by the fact that its successful production has often been linked with the stability of the world (Palacpac, 1982).

Wetland rice culture and its associated soil characteristics: Rice produces greater yields when it is grown in flooded vs unflooded soil (DeDatta 1981). Submergence results in greater suppression of weed growth; higher efficiency in the utilization of added nitrogen; and better insect and weed control with grannular chemicals. In south and

southeast Asia, 95 percent of cultivated rice areas are in wetland rice culture (Barker and Herdt 1979). The general operations involved in submerged rice cultivation are:

(1) Submergence of the soil, with or without puddling, for the duration of the crop and with or without soil drying in midseason;

(2) Draining and drying the soils before harvest; and

(3) Reflooding for the next crop a few weeks to several months after harvest.

These wetland practices result in greater than 95% of the rice growth cycle being completed while it is submerged. Since oxygen diffuses 10,000 times slower through water than through a gas phase (Greenwood and Boodman 1964), oxygen availability in submerged soils is very low. Oxygen that is trapped or diffuses into soil is rapidly consumed by microbial respiration. Under the resultant anaerobiosis, facultative and strict anaerobes predominate the microbial community in submerged soils (Hayashi et al. 1978). The major anaerobic microbial processes occurring in submerged soils are illustrated in Table 1 (Tiedje et al. 1984). Soil constituents NO_3^- (and other nitrogenous oxides), Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2 and H^+ ions serve as electron acceptors and are reduced, sequentially according to their thermodynamic yield to N_2, Mn^{2+}, Fe^{2+}, S^{2-}, CH_4, and H_2 respectively. However, even in a permanently flooded soil, a range of reducing conditions exist with depth in the soil (Sethunathen et al. 1982). A flooded rice field is characterized by four distinct zones: flood (standing) water; a thin (few millimeters) oxidized surface soil layer; reduced soil layer; and the rhizosphere (Yoshida 1975, Sethunathen et al. 1982, Watanabe and Furusaka 1980). Major microbiologically mediated processes in flooded

Table 1. Anaerobic microbial processes and their reaction products (Tiedje et al. 1984).

Process	Reaction[a]
Fe^{3+}, Mn^{4+} reduction	OM[b] + Fe_3^+, Mn^{4+} → Fe^{2+}, Mn^{2+}
Denitrification	OM + NO_3^- → N_2O, N_2
Fermentation	OM → organic acids, principally acetate and butyrate
Nitrate respiration	OM + NO_3^- → NO_2^-
Dissimilatory NO_3^- reduction to NH_4^+	OM + NO_3^- → NH_4^+
Sulfate reduction	OM or H_2 + SO_4^{2-} → CH_4, acetate
Acetate splitting	Acetate → CO_2 + CH_4
Proton reduction	Fatty acids and alcohols + H^+ → H_2 + acetate + CO_2

[a] The major reduction products are shown. Oxidized products are also produced; this is usually CO_2 if the electron donor is an organic compound.

[b] OM = Organic matter.

rice soils are:

(1) Nitrogen fixation by algae and bacteria, and nitrification in the flood water.

(2) Methane and ethylene oxidation, nitrification, sulfide oxidation, ammonification, nitrogen fixation and methane oxidation in the surface oxidized layer.

(3) Nitrogen fixation, ammonification, denitrification, Fe^{2+} and Mn^{2+} reduction, dissimilatory nitrate reduction, sulfate reduction, and methane production in the reduced soil layer.

(4) Nitrogen fixation, sulfide oxidation, nitrification, denitrification, and oxidation of Mn^{2+}, Fe^{2+}, and methane in the rhizosphere.

These oxidation and reduction reactions in flooded rice soils are responsible for organic matter mineralization and the recycling of mineral nutrients.

Sources of organic matter in flooded rice soil: In rice growing areas, soil organic matter is supplied by rice straw, husks, root exudates, and senescent roots. A large proportion of organic matter in rice fields of China are derived from green manures, mainly milk vetch (Lin 1982). Bluegreen algal biomass also contributes to the total organic matter reaching paddy soils. However, their major contribution is through nitrogen fixation carried out alone or in symbiosis with the water fern azolla. The amount of rice straw produced in paddy conditions is a function of water regime, season, cultivar, soil fertility and the grain-straw ration (Ponnamperuma 1984). Alleviation of growth-limiting factors to rice increases the grain and straw yields.

Tall cultivars grown during the wet-season produce more straw than short cultivars in unfavorable seasons (Palacpac 1982). The grain/straw ratio ranges from 0.57 during the dry season to 0.89 during the wet season. A straw production of 15.2 $t \cdot ha^{-1}$ was recorded with the Meeung Naung 62M variety (Ponnamperuma 1984). Rice grain production was 470 million tons in 1984 (FAO Yearbook 1985). If one assumes a grain/straw ratio of 0.7, 320 million tons of rice straw was produced in 1984. On the average rice straw contains 41.3% total carbon, a C/N ratio of 51.0, a crude ash content of 18.8%, hot-water extractable organic matter of 11.6%, and 7.7% lignin (Inoko 1984). Among the organic compounds produced, the carbohydrate fraction contains 20.6% hemicellulose and 24.7% cellulose. In addition to rice straw, the plant roots, stubble, sloughed root cells and exudates contribute to the organic matter of flooded rice soils (Ward 1974; Dommergues and Rinaudo 1979). The contribution of these various sources of organic matter to the total amount of microbially processed carbon and minerals is however poorly documented in the literature.

Organic matter decomposition in flooded rice paddies: The potential microbial processes involved in organic matter decomposition in flooded rice soils are illustrated in Figure 2. Due to the lowered availability of oxygen in submerged soils rice straw is decomposed primarily by fermentative bacteria. Fermentation metabolites, (e.g., acetate, propionate, butyrate, lactate, hydrogen and carbon dioxide) can be oxidized to CO_2 through aerobic and anaerobic respiration, or to CH_4 either through CO_2 reduction or acetoclastic methanogenesis. The rates of the various processes are controlled by the availability of the

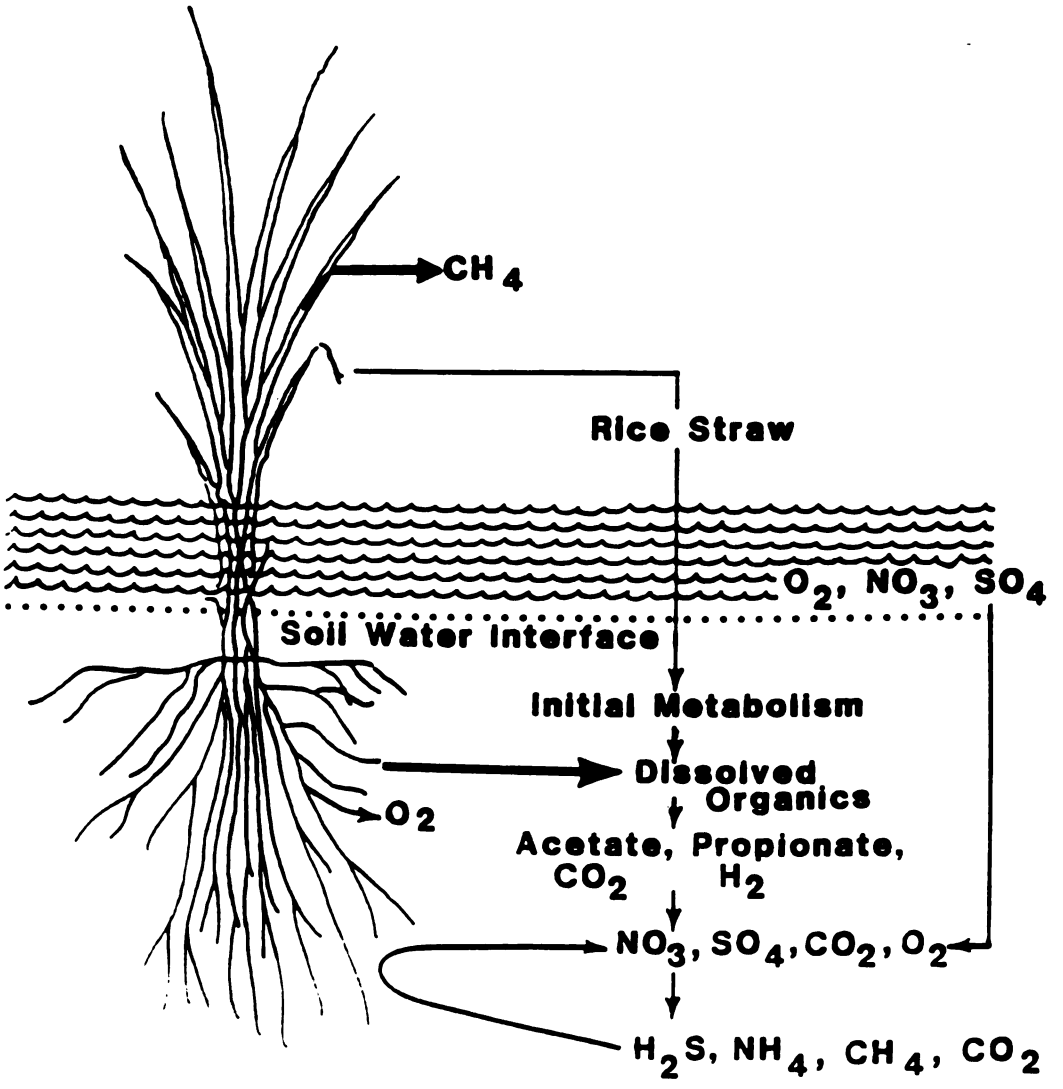

Figure 2. Microbial processes and plant interactions in paddy soils.

appropriate electron acceptors such as O_2, NO_3^- and SO_4^{2-} or HCO_3^-. The availability of electron acceptors depends on 1) the rate of diffusion of electron acceptors to the site of consumption; 2) the rate of diffusion of products out of the anaerobic zone and their subsequent reoxidation; 3) the demand for electron acceptors. When the rate of regeneration of electron acceptors or the rate of diffusion can not meet the demand for electron acceptors, fermentation and methanogenesis become dominant processes in organic matter metabolism in submerged soils. In flooded rice soil the overlying water column, the oxidized surface soil layer and the rhizosphere are sites of the generation or regeneration of inorganic electron acceptors. However, rice plants also utilize NO_3^- and SO_4^{2-} for growth and are therefore in competition with microbial processes for these ions. Furthermore, rice plants contain large contiguous internal air spaces which transport atmospheric O_2 to the roots (Arashi and Nitta 1955; Barber et al. 1962; Armstrong 1969, 1978, 1979; Armstrong and Webb 1985). Potentially a portion of the O_2 diffuses into the rhizosphere and functions as an electron acceptor.

Since anaerobic metabolism in natural systems is partially regulated by the ability of the microbial community to consume reducing equivalents released during the oxidation of organic matter, it is important to understand the major anaerobic processes involved in organic matter decomposition which relate to methane production in and emission from flooded rice soil.

The goals of this study were to: (1) investigate the changes and distributions of inorganic ions (NO_3^-, SO_4^{2-}, NH_4^+) and short-chain volatile fatty acids after dry soil was flooded and planted with rice; (2) to measure the potentials for nitrification, denitrification, and

sulfate reduction in flooded soils, (3) to examine the influence of rice plants on methane production and emission from flooded rice soils, and (4) to investigate the influence of continuous rice cropping, straw incorporation and N-fertilizer application on methane production in and emission from flooded paddy soils.

To accomplish these goals the study was divided into two major experiments. Experiment one was designed to examine the affect of the plant and its phenology on the microbial processes in never planted experimental paddy soils. Experiment two examined the same questions, in harvested soils (containing roots) from experiment one. Other soils used in experiment two were further amended with either fertilizer and/or rice straw.

MATERIALS AND METHODS

Experiment One

Experimental design and pot preparation: Pots used in all experiments were 10 gallon PVC nursery pots (Molema Nurseries, Akron, Ohio) lined with 6 mil polyvinyl plastic bags. A subsoil irrigation system for initial internal flooding and gas displacement consisted of a perforated 1/2" PVC pipe on the bottom of the container connected to a vertical nonperforated 1/2" PVC pipe to the surface. Four sampling cylinders were placed vertically in the pots as illustrated (Figure 3). The cylinders consisted of polycarbonate tubing (1.25 inch ID, 1.37 inch O.D., 12 inch long) having holes (0.25 inch) drilled over the entire length and circumference. The tubes were covered with Nitemesh (Nitex

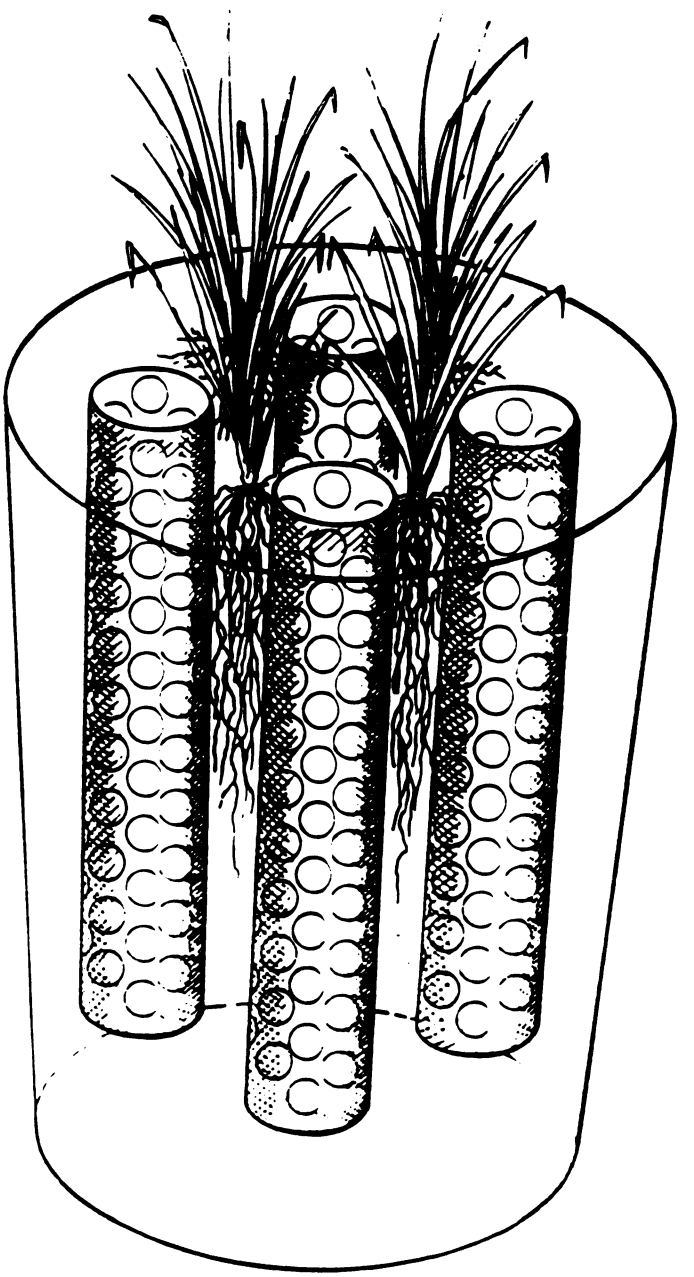

Figure 3. Experimental pots and sampling cylinders.

mesh No. 305T) which had a mesh opening of 48 μm and an open surface of 33%.

Kalamazoo sandy loam (pH. 7.4, organic matter content 3.0%, CEC 15 meq/100g soil) was used in all studies. The soil was initially crushed and sieved through a 2-mm-mesh screen followed by complete mixing in a rotary cement mixer to assure homogeneity. The processed soil was placed in the pots and gently settled to a depth of 12 inches. The same soil was placed in the sampling cylinders. The cylinders allowed for the taking of soil core samples during the growth of the rice without damaging the roots, which encircled but did not penetrate the mesh.

Soils were initially flooded through the sub-surface irrigation system with deionized water to displace air from pore spaces with the rising water. Subsequent irrigation consisted of the manual addition of deionized water from the surface to maintain a water depth of 2.5 inches.

Rice seeds (Oryza sativa cv An-nan-chiao, type japonicum) were germinated in petri dishes on moistened filter paper. Plates were maintained at 80°F and exposed to a 14 hour light and 10 hour dark cycle. Germinated seeds were placed in seedling trays containing the previously described soil. The soil was flooded with deionized water and the seedlings were maintained under the conditions described above for 14 days.

Four weeks after dry soils were flooded, four hills (each hill contains 3 plants) of 18-day old rice seedlings were transplanted into the experimental pots with a spacing of 6.5" x 6.5" (Figure 3) in the pot. No fertilizer application was made during the rice growing period; however, 1.5 mmoles of EDDHA (Sequtrene 138) was applied to correct Fe

deficiencies during the seedling and early growth stages. Greenhouse conditions were: a temperature 80-75°F Day-night cycle, relative humidity 40-60%, and supplemental lighting to provide a light cycle of 14h and a 10h dark cycle. Pots were placed 6 inches apart in rows which were 3 ft. apart. Replicates of the various treatment were randomized among the controls.

Soil solutions sampling: Soil pore water samples were taken using a suction microlysimeter at various depths of soil, e.g. 0, 5, 10, 15, and 25 cm every three days from the beginning of the flooding of dry soil to the maturing stage of rice.

Microlysimeters consisted of a 25-cm-long, 18 ga. needle which had 0.25-mm-diameter holes drilled into the distal centimeter. The perforated end was covered with Porous teflon tubing (PTFE, Gore Manufacturing, Cleveland, Ohio). The hydrophobic membrane was "wetted" in 95% ethyl alcohol rinsed in deionized water and connected to a syringe which was used to withdraw the samples.

Soil pore water samples were also taken from cores obtained from the sampling cylinders at two week intervals. Cores were removed by hand coring in 1" O.D. x 7/8" ID. acrylic plastic core tubes. Cores were extruded and sectioned at 2 cm. intervals, followed by centrifugation at 1,000 g.

All soil solutions taken from the experimental treatments were filtered through 0.2 µm Nucleopore membrane filters prior to analyses.

Measurement of methane emission: Rates of methane emission were determined by measuring the increases of methane concentration in a dome placed over the water surface of the experimental pot. The dome, a

48-liter bell jar with a septum in the top, was placed on the edge of the pot to avoid disturbing the soil surface, which would potentially release entrained gas bubbles. An additional enclosure consisting of a wire frame 13 inches in diameter by 32 inches high covered with a Saran plastic bag (Anspec Inc., Ann Arbor, MI) was used in the latter growth stages and in field measurements. A twenty-five ml gas sample was taken by using a glass 50-ml syringe and needle through a stopper fitted on the top of the dome. The withdrawn gas sample was transferred into an evacuated serum bottle stoppered with a butyl rubber stopper (Bellco Glass Inc., Vineland, NJ) and brought back to the laboratory for analysis.

A smaller 1-liter dome consisting of a 6" glass pipe cap with a hole drilled through the top to accept a septum was used to measure methane flux across the water-air interface between plants.

Plant biomass: Two weeks after transplanting, two rice pots (8 plants) were sacrificed each week in order to observe changes in plant biomass over the experimental period. Plants were cut at ground level and roots were collected and washed. Roots were sectioned at 4 cm. intervals to determine the percent of the total root biomass found with depth. The samples were dried at 60°C in a forced air oven until a constant weight was obtained.

Chemical Analyses

Nitrate and nitrite determination: High pressure liquid chromatography (HPLC) was used for the analyses. Separation of NO_2^- and NO_3^- was accomplished by eluting the sample through a Whatman PX-10 column with 50 mM phosphate buffer solution (pH 3.0) at a flow rate of

2.0 ml/min. Peak detection was on a Shimadzu SPD-6A U.V. detector (Shimadzu Corp., Columbia, MD) at 210 nm. The retention times for nitrite and nitrate were 3.2 and 3.9 min., respectively. The signal from the detector was processed with a Shimadzu C-R 3A integrator.

Sulfate determination: Sulfate was analyzed using (HPLC) and was separated on a Vydac anion exchange chromatography column (Vydac 302-2C, 25 cm long), eluted with 0.002 M phthalic acid buffer adjusted to pH 4.9 with anhydrous sodium borate. A flow rate of 2.5 $ml \cdot min^{-1}$ yielded a retention time for sulfate of 7 min. Sulfate was detected with a conductivity detector (Anspec Inc., Ann Arbor MI). A Shimadzu C-R,3A integrator was used to process the signal from the detector.

Organic acid determination: Organic acids were separated on a HPLC Biorad HPX-87 column (30 cm x 4.1 mm) eluted with 0.007 N H_2SO_4 continuously purged with helium gas, at a flow rate of 0.6 $ml \cdot min^{-1}$ and column temperature of 60°C. The fatty acids lactate, fumarate, succinate, acetate, propionate, butyrate, and isobutyrate were separated and detected at 210 nM with a Shimadzu SPD-6A U.V. detector. A Shimadzu C-R,3A integrator was used to analyze the recorded signal from the detector.

Ammonium determination: NH_4^+ was determined spectrophotometrically, using the indophenol-blue method of Koroleff (1972). Three reagents were used to develop the indophenol-blue. Reagent A consisted of a citrate buffer containing 66.7 g trisodium citrate dihydrate, 34 g boric acid, 19.4 g citric acid and 3 g sodium hydroxide per liter of ammonium free water (AFW). Reagent B contained 35.0 g phenol and 0.4 g sodium

nitro ferric cyanide per liter of AFW. Reagent C was prepared by dissolving 20 g sodium hydroxide in 5 mls of AFW, followed by the addition of 2.0 g sodium dichloroisocyanurate, and diluting to 1 liter with AFW. The procedure for NH_4^+ determination was as follows: 10 ml of sample (or sample dilute to 10 mls with AFW) were transferred into 11 x 18 tubes followed by the addition of 1.2 mls Reagent A, 0.3 mls Reagent B and 0.45 mls Reagent C. Addition of the reagents was done quickly and the samples shaken immediately. Samples were allowed to stand in the dark for a minimum of 8 hours but not in excess of 48 hours. Absorbance was read at 630 nm in 1 cm cell on a Hitachi spectrophotometer (Model 100-40).

Gas analysis: Analysis of carbon dioxide, oxygen, nitrogen, and methane was achieved with a Carle BGC-8500 (Carle Industries, Sunnyvale, CA), equipped with a 1 meter (1/8" dia) Poropak Q and a molecular sieve 5A columns (Carle Industries, Sunnyvale, CA), connected in series to facilitate separation of the component gases. Helium was used as carrier with a flow rate of 30 $ml \cdot min^{-1}$ and an oven temperature of 60°C. Carbon dioxide and H_2O were separated from other constitute gases on the (Porapak Q) column. A valving arrangement (Carle Industries) allowed those gases to bypass the downstream column (molecular sieve 5A 80/100) where O_2/Ar, N_2, CH_4 were separated. The separated gases were passed directly to the thermister detector. Gas mixtures (Linde Corp, Chicago, Ill.) were used as standards. A Shimadzu CR-3A integrator was used to record the signal from the detector. Concentrations of CH_4 were analyzed on a Varian 3700 gas chromatograph (Varian Inst., Sunnyvale, CA.) equipped with a flame ionization detector (FID). Methane was

separated on a 2-m (1/8" dia.) poropak N Column at an oven temperature of 50°C using helium as a carrier gas at a flow rate of 20 $ml \cdot min^{-1}$. Methane standards from 5000 ppm to 2 ppm were prepared from 100% methane using Helium as a diluent gas. A Shimadzu CR-3A integrator was used to record the signal from the detector.

Nitrous oxide was analyzed on a Varian 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD). Gas samples were injected into a 250 µl loop and separated on a 2.5 m long (1/8" dia.) Porpak Q column using P-10 gas mixture (10% CH_4 in Argon) as carrier. The flow rate was 12 $ml \cdot min^{-1}$ and oven temperature 50°C. The retention time was 4.3 min. Standards were prepared from 55 ppm standard (Linde Corp.) and pure N_2O using Helium as the diluent gas.

Rate Measurements

Potential rates of nitrification: A modified technique of Belser and May (1980) was used to examine potential rates of nitrification. Soil cores were taken from the experimental treatments, sectioned at 3 cm intervals and homogenized with 50 ml of ammonium phosphate buffer (1 mM potassium phosphate, pH 7.2; 0.25 mM $(NH_4)_2SO_4$) in a 125 ml Erlenmeyer flask. The flasks were covered with aluminum foil and incubated at 22°C with vigorous agitation (200 revolutions per min) on a gyrotory shaker (New Brunswick, Edison, NJ). Portions of the incubation suspension were withdrawn at various time intervals, filtrated through 0.2 µm Nucleopore filter and analyzed for NO_2^- and NO_3^-. Increase in concentration over time was used to calculate nitrification potential.

Potential rates of denitrification: Nitrous oxide reductase activity was assayed and used as an estimate of denitrification capacity

(Garcia 1973). Soil cores were taken from experimental pots as described above, subcored through the center of the section with a 5-ml syringe which had the needle hub cut off in order to minimize exposure to oxygen. The subcored soil sample (4-ml) was transferred into 35-ml serum bottles which were previously flushed with oxygen-free nitrogen and sealed with a butyl rubber stopper (Bellco). The serum bottle was again flushed with oxygen-free nitrogen for 3 min. and 50 µl of a N_2O solution (prepared by applying 50 kPa of 10% N_2O in the head space of the dilution bottle) was added with a Hamilton microsyringe and needle. The bottle was shaken vigorously for one minute and incubated at 22.5°C. Change in the concentration of N_2O in the head space was measured at 4 hour intervals using a Varian 3700 gas chromatograph equipped with ^{63}Ni electron capture detector described above. The rate of N_2O disappearance was calculated as the denitrification potential.

Sulfate reduction: The rate of sulfate reduction was estimated by directly measuring the depletion of sulfate concentration in pore water of soils incubated in sealed serum bottles. Soil cores were obtained from the various treatments and handled as described above. Pore water was obtained over time with microporous lysimeters, and changes in sulfate measured as described for sulfate determination.

Methane concentration and rates of methane production: Soil cores were obtained from experimental pots as described above and subsamples extruded into 35 ml serum bottles previously flushed with O_2-free nitrogen, and stoppered with butyl rubber stopper. Three bottles were autoclaved for 15 min to stop methane production and drive methane from the soil solution. The gases (0.5 ml) in the head space were withdrawn

and 0.3 ml injected into a Varian 3700 gas chromatograph equipped with flame ionization detector (FID). Methane concentration was estimated by head space volume, amount of soil used and water content. Bottles containing soils for estimating methane production were evacuated and filled with an O_2-free gas mixture (83% N_2, 7% CO_2 six times to reduce the ambient methane concentration and incubated (22.5°C). Methane production rates were measured at one hour intervals for 8 hours, by examining changes in methane concentrations in the head space.

Respiratory index: Radio labeled 2-^{14}C acetate (54 mCi/m·mole; New England Nuclear Corp.) was used. The method used was after that described in Lovley and Klug (1982). Briefly, soil cores were obtained from an experimental treatment as described previously; subcored with 3-ml syringes with the needle hub cut off and the open end of the syringe sealed with a butyl rubber stopper (Bellco). A 50 µl aliquot of 1-^{14}C-acetate (containing 1 µCi of ^{14}C) which had been preflushed with oxygen-free nitrogen was directly injected into subcores with a 250 µl Hamilton syringe. The syringe needle was inserted through the serum stopper and the labeled acetate was injected as the needle was withdrawn. The injected subcores were incubated at (22.5°C) for appropriate time intervals. The incubation was stopped after injection by immersing the subcores in liquid nitrogen (0 hour) followed by freezing in -80°C freezer. Prior to analyses the surface of the subcores was warmed to allow the cores to be extruded into 60 ml serum bottles containing 2 ml of 4 N glutaraldehyde which had previously been flushed with O_2-free nitrogen. Samples were allowed to thaw in the bottle, samples shaken and the head space sampled. Gas samples (1.0 ml)

were analyzed for specific activities of $^{14}CH_4$ and $^{14}CO_2$ with a Varian 3700 gas chromatograph equipped with thermal conductivity detector that was connected in series with a gas proportional counter. Gases were separated at 50°C on a 3M, 1/8" dia. column packed with Porapak N (100/120 mesh, Waters Associated) with a helium flow rate of 20 ml/min. The injector and detector temperature were 120°C and 160°, respectively. $^{14}CH_4$ and $^{14}CO_2$ were eluted with retention times of 1.4 and 7.0 min. and data recorded on a Shimadzu (C-R 3A) integrator. The Respiration Index was calculated as: $RI = {}^{14}CH_4/({}^{14}CH_4 + {}^{14}CO_2)$.

Experiment Two

Experimental design and pot preparation: At the conclusion of experiment one, one set of experimental pots used in experiment one were drained and maintained at field capacity for one week. Rice straw produced in experiment one was cut into 4 cm long pieces and incorporated by raking into the top 3 cm of soil at a rate equivalent to 45kg ha^{-1}. Pots were flooded and kept in a flooded condition until time of planting. Another set was maintained in a flooded state (without straw addition) until time of transplanting. Rice plants used, transplanting, water management techniques, and environmental conditions were the same as for experiment one. Rates of production in and emission from experimental soils were measured every 1-2 weeks after rice transplanting.

Effect of nitrogen fertilizer application: Ammonium sulfate or ammonium chloride was applied at an equivalent rate of 50 kg N ha^{-1} to examine the affect of the application of nitrogen fertilizer on the production and emission of methane. Four treatments and three

replications were involved in this experiment. Treatments were: A) Ammonium sulfate addition; B) Ammonium chloride addition; C) Ammonium chloride plus ferrous sulfate addition; D) No-nitrogen addition.

In treatment C, the same amount of sulfate applied in Treatment A was added by using 269 kg ha^{-1} of $FeSO_4$. Rice transplanting and management were the same as described in experiment one. The fertilizer treatments were applied one day before rice transplanting. Methane production and emission from experimental pot were determined at rice flowering and maturing stages beginning 50 to 70 days after rice transplanting.

RESULTS AND DISCUSSION

Sampling techniques: In order to collect pore water without disrupting conditions in the experimental pots, the micro-suction lysimeter described in the materials and methods was used. In order to validate the use of this method pore water concentrations of chemical constituents such as nitrate, sulfate and short chained volatile fatty acids at different soil depths were compared to those obtained with conventional methods. The latter consisted of coring the soils, sectioning, and centrifugation of sections at 1000 G to obtain pore water for analyses. Results are illustrated in Table 2a and 2b. Concentrations of NO_3^-, SO_4^{2-} and short chain volatile fatty acids (VFA's) in pore waters collected by the coring procedure were not significantly (5% level) different from those taken by microporous suction lysimeters and standard core sectioning method. Howes et al.

Table 2a. Comparison of nitrate and sulfate concentrations (μm/l) in soil solutions which were taken by different sampling methods.

Depth of Soil	Suction microlysimeter		Conventional method	
cm	NO_3^-	$SO_4^=$	NO_3^-	$SO_4^=$
0- 4	1546.7 (137.2)*	1201.0 (8.89)	1399.0 (378.2)	1139.3 (320.4)
4- 8	82.3 (42.0)	955.0 (247.7)	120.0 (28.2)	1399.0 (189.9)
8-12	12.7 (9.3)	560.0 (289.2)	19.0 (8.5)	1017.0 (403.3)
12-16	3.7 (1.5)	227.7 (182.5)	7.7 (3.2)	494.0 (181.5)
16-20	**	**	6.0 (5.5)	210.0 (199.5)
20-24	3.5 (0.6)	73.7 (17.5)	5.0 (1.0)	35.3 (2.31)

* Mean followed by the standard deviation

**Not detected

Table 2b. Comparison of pore water acetate and propionate concentrations (μm/l) taken by different sampling techniques

	Acetate		Propionate	
	Suction microlysimeter	Conventional method	Suction microlysimeter	Conventional method
0-4	**	**	**	**
4-8	232(20.8)*	203(30.3)	**	**
8-12	442(40.0)	476(32.2)	63(10.2)	75(20.2)
12-16	392(19.0)	463(45.6)	142(20.0)	166(15.9)
16-20	**	586(30.8)	**	176(33.8)
20-24	493(35.5)	504(26.6)	107(30.7)	129(40.1)

*Mean followed by standard deviation

**Not detected

(1985) found the core sectioning technique in salt marsh soils to significantly increase the concentration of total dissolved organic carbon and dimethylsulfide, due to root damage, while other soil constituents such as NO_3^- and SO_4^{2-} were not significantly different in soil solutions obtained by different methods. The former would presumably stimulate the rates of processes using dissolved organic matter. In our experimental system, rice roots were separated from the soil with the previously described porous cylinders which allowed core removal without root damage. Pore water NO_3^-, SO_4^{2-} (Table 2a) and VFA concentrations (Table 2b) were not significantly (5% level) different in samples taken by microporous suction lysimeters and from cores taken from inside the cylinders by the standard core sectioning method. Considerable heterogeneity occurred in the profiles of sulfate. Jørgensen (1978), heterogeneity such as this can be attributed to the patchiness of sulfate reduction within sulfate containing zones.

Plant biomass: Changes in the above and below ground biomass at various times after transplanting are illustrated on Figure 4. The same general pattern of increase was observed for the above and below ground biomass during various stages of growth with no increase in either after flowering (50 days after transplanting). Above ground biomass was greater than double that of the below ground biomass at any of the sampling dates. Similar growth patterns have been reported for field grown rice (Yoshida 1981). The standard deviation between plants was small which probably reflects the homogeneity of the soil and conditions associated with the experimental treatment.

The percentage of root biomass at various depths in the soil at

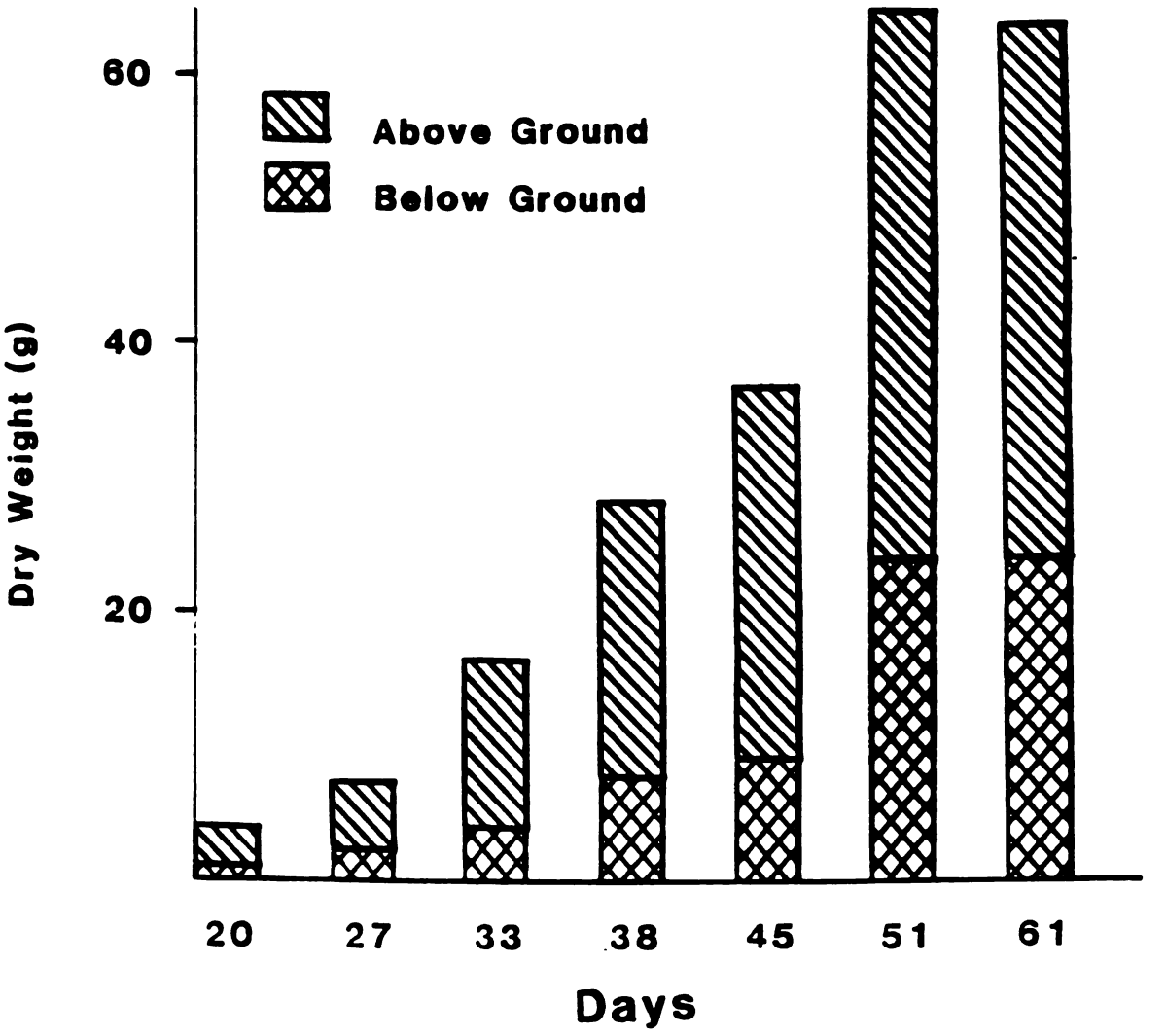

Figure 4. Above and below ground plant biomass during the growth period.

panicle initiation (30 days) and maturing phase (50 days) is illustrated in Table 3. In both cases the maximum root biomass was observed in the top 4 cm, and a steep decrease in biomass below this depth interval. This relationship is also noted in field grown rice (Yoshida 1981).

Table 3. Percent distribution of root biomass at panicle initiation and maturing stage of growth.

Depth	Panicle Initiation (30 days*)	Maturing (50 days*)
0- 4	75 ± 4	83 ± 6
4- 8	15 ± 2	10 ± 3
8-12	41 ± 0.8	3 ± 2
12-16	31 ± 1	2 ± 2
16-20	1.8 ± 2	1.2 ± 2
20-25	1.2 ± 1	0.8 ± 3

*days after transplanting

Nitrogen Transformations

Nitrogen containing fertilizers are often added to paddy soils to increase yields of rice. Nitrogen is considered to be the most important nutrient of the normal fertilizer elements (N, P, K) added in paddy soil (Dommergues et al. 1979). In flooded rice soils the addition of ammonium sulfate followed by ammonium chloride is recognized as the most efficient N-fertilizer. Nitrate containing fertilizer is not recommended in flooded soil conditions due to inevitable losses through denitrification or leaching.

Ammonium produced through organic matter mineralization is either consumed by the plant or is oxidized through nitrification at the root surface or in the oxidized layer of flooded soils. The products of nitrification NO_3^- and NO_2^- are used by denitrifiers as electron acceptors, and reduced to N_2 or N_2O depending on the species of denitrifiers and concentrations of the reactants (Tiedje et al. 1984).

Distribution of NO_3^-: In order to estimate the rate of denitrification, pore water concentrations of NO_3^- were followed at 4 day intervals after the flooding of dry soil. The changes in the concentration of NO_3^- in the planted and nonplanted treatments over the experimental period are illustrated in Figure 5. Initially nitrate concentrations in pore water were high after the flooding of the dry soil. This initial high concentration is thought to reflect the concentration present in the agricultural soils at the Kellogg Biological Station. It is also possible that NH_4^+ present in these soils may have been nitrified during the period between when the soil was dug, stored and sieved.

Nitrate concentration in planted treatments was higher, remained longer and increased in concentration after planting as compared to that in the nonplanted treatment. Nitrate concentration in nonplanted flooded soil (Figure 5) rapidly declined following 12 days of flooding. The profile of depletion with depth exhibited the same general pattern over the first 40 days after which no NO_3^- was detected. The results agree with the data of MacRae et al. (1968) who demonstrated that 59-100% of added $^{15}NO_3$ disappeared with 6 weeks of its incubation in six different submerged soils.

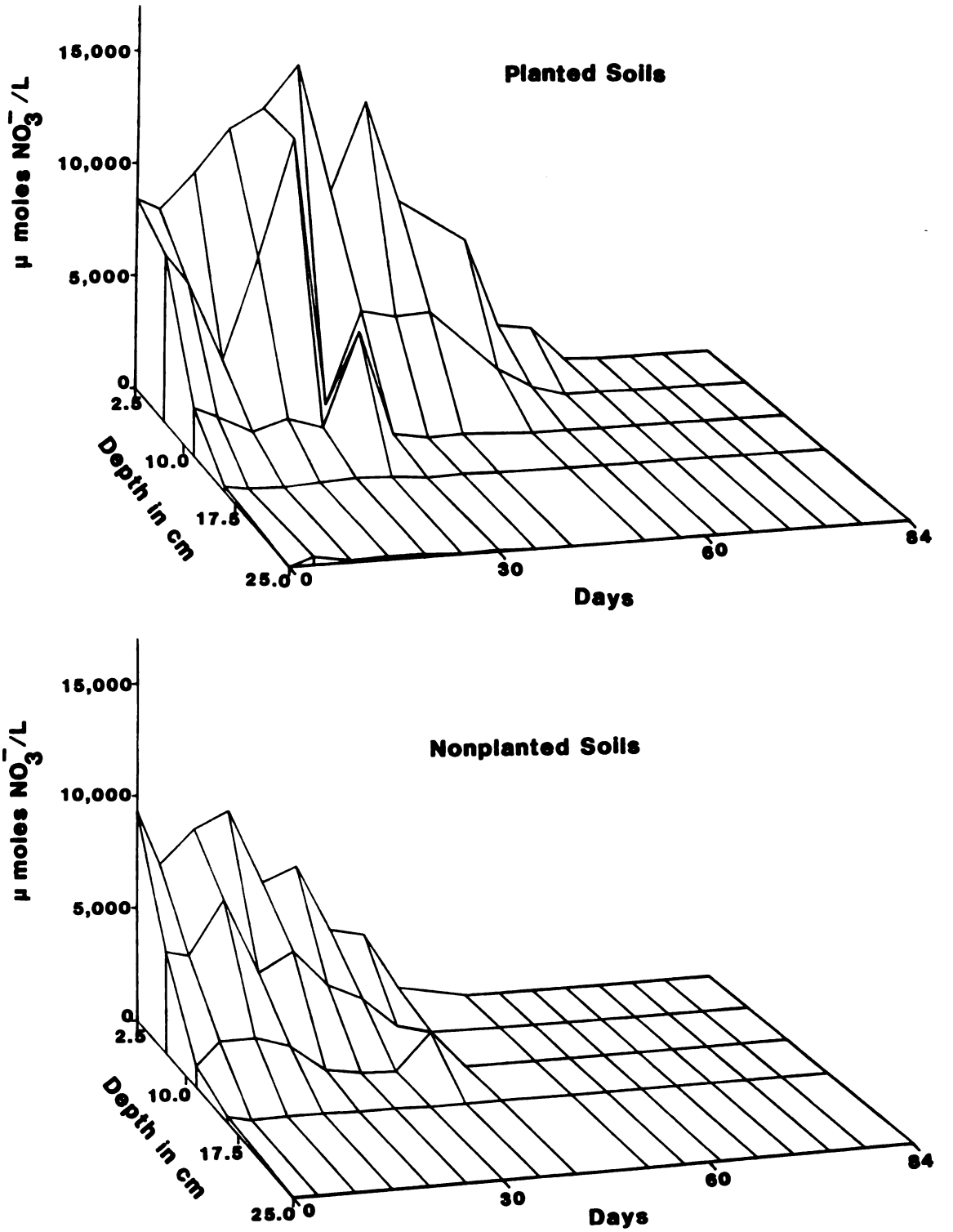

Figure 5. Depth-time distribution of nitrate in planted and nonplanted soils at 0, 30, 60 and 84 days of the experiment.

The higher NO_3^- concentration observed in the planted soils may be explained by increased ammonification driven by plant exudates or senescent root cells followed by nitrification of the released ammonia in the rhizosphere or surface soils. Interestingly the ammonia concentrations (Figure 6) in the planted vs the nonplanted treatment are very similar suggesting that nitrification may have been inhibited in the nonplanted treatment by the lack of oxygen which would be produced by the plants. This is supported by the highest root biomass (Table 3) in this zone. Smith and Delaune 1984 report a similar effect of rice plants on nitrification in the root zone. The process of O_2 transport through lysigenous channels in the roots and partial loss to the rhizosphere has previously been shown to modify conditions in the rhizosphere (Bristow 1975; Armstrong 1978; Trolldenier 1988). Another factor which may be responsible for increasing oxygen concentrations in surface sediments is downward movement of oxygenated surface waters due to transpirational loss of water from the plant (Dacey and Howes 1984). Although evaporation vs transpirational loss of water was not estimated, it was noted that water addition to planted treatments was approximately twice that added to the nonplanted treatment during the active growth period. This suggests that downward water movement was occurring and should be considered in future studies of this nature. The depth profiles of NO_3^- depletion are steeper in the planted treatment and the NO_3^- remained in surface sediments 2 months after flooding. The steepness of the profile in surficial soils also supports the suggestion that increased NO_3^- is produced in these surface soils due to the presence of O_2 which would increase rates of nitrification and decrease rates of denitrification.

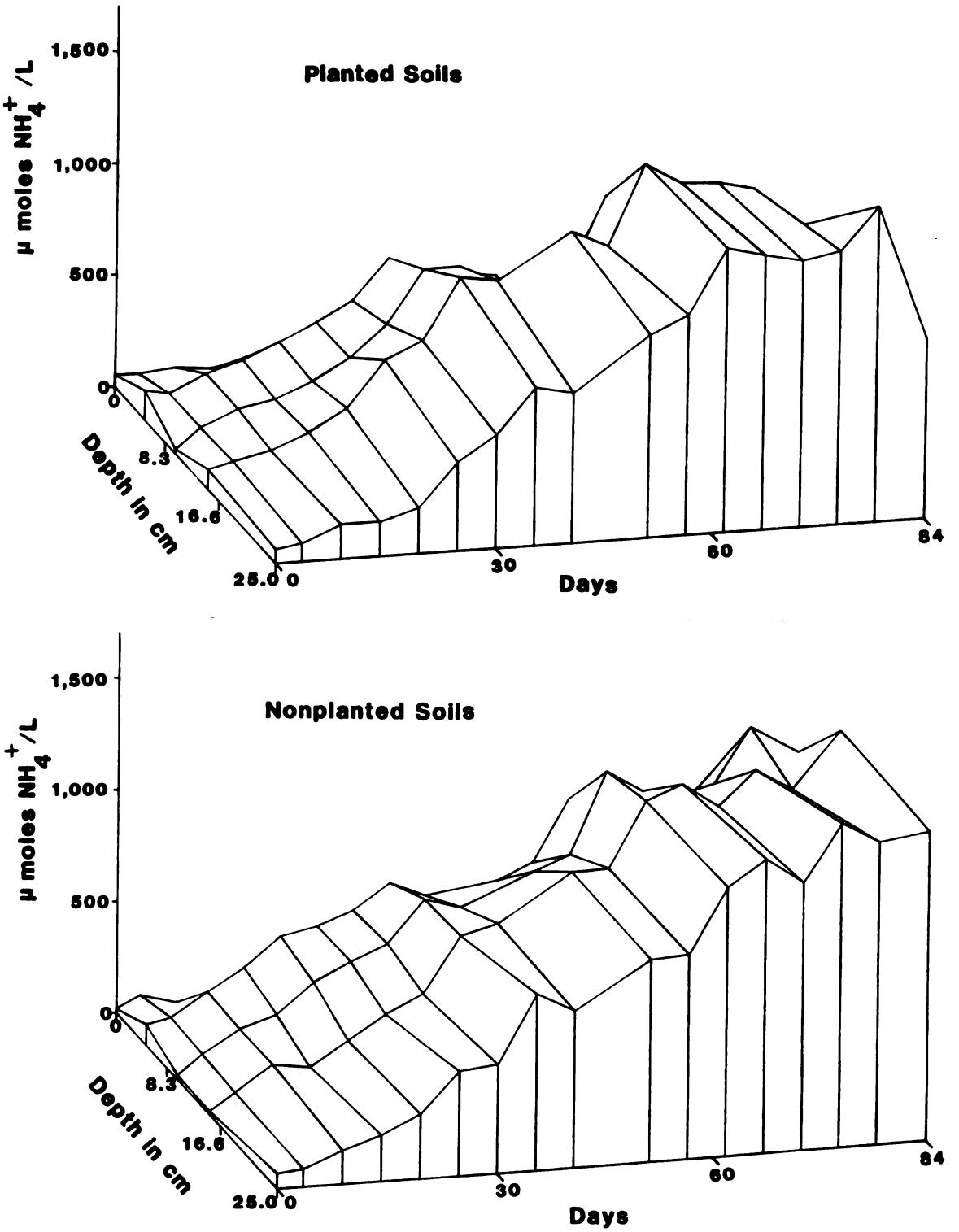

Figure 6. Depth-time distribution of dissolved ammonia in planted and nonplanted soils at 0, 30, 60 and 84 days of the experiment.

Distributions of dissolved NH_4^+: Distributions of dissolved NH_4^+ in planted and nonplanted soils at different depths over the treatment period is illustrated in Figure 6. The ratio of dissolved to total ammonia in planted soils taken at 40 days after flooding (i.e. dissolved plus adsorbed) is illustrated in Table 4. Dissolved ammonia levels ranged from 9-22% of the total, indicating that the majority of NH_4^+ is bound to soil particles. It is not known what the ammonia sorption isotherms are for these soils when maintained under these conditions or whether the ratio changes at various stages of growth. In both treatments NH_4^+ concentrations increased during the first 40 days of the treatment period. Following this time the concentration leveled off in the planted treatment but continued to increase in the nonplanted treatment. Concentrations were consistently higher in the deeper portions of the profile, with the lowest concentrations being in surface soils. These profiles are the inverse of those found for NO_3^- (Figure 5) suggesting a relationship between the processes controlling the concentration of the two ions. The leveling off of the concentrations of NH_4^+ in the planted treatment especially in the zones of highest root biomass (0-10 cm) suggest that the more oxidized environment stimulates nitrification in this zone and/or the plant is taking up NH_4^+ which is the preferred nitrogen source of the plant. This type of relationship was previously reported by Ando et al. 1983. As pointed out above this is the zone where the concentration of NO_3^- is the highest which would correlate with increased nitrification in this zone. It would appear that nitrification and plant uptake would be in competition with each other, however it is not known what the rate limiting concentration is for both of these processes (Broadbent and Reyes 1971).

Further studies should examine changes in the adsorbed to dissolved ammonia ratio since changes in redox overtime could markedly change this relationship (Broadbent and Nakashima 1970; Reddy and Patrick 1986). The effect of redox differences is suggested by the lowest percentage of dissolved ammonia (Table 4) in the deepest zone where the redox would be expected to be the lowest.

Table 4. Depth distribution of dissolved and exchangeable NH_4^+ in planted soils at day 40 of experiment 1.

Soil depth (cm)	Dissolved NH_4^+ (μM)	Exchangeable NH_4^+ (μM)	$\frac{\text{Dissolved } NH_4^+}{\text{Total } NH_4^+} \times 100$
2	0.44	4.10	18.3
6	1.30	6.65	22.3
10	1.81	7.43	18.6
14	2.15	9.40	19.6
18	2.51	8.72	16.6
22	2.20	9.81	9.6

Nitrification potential: Nitrification potentials were compared in planted and nonplanted treatments 30 days after transplanting. This was done to further examine the source of the observed NH_4^+ and NO_3^- profiles Figures 5 and 6, which could be as a result of differences in rates of nitrification. Table 5 illustrates that the highest nitrification rates occurred in the top 3 centimeters in both treatments, however no nitrification was observed in depths below this

in the nonplanted treatment. Nitrification potential within the upper 3 cm of soil was 0.167 μm NO_3^--N/g soil/hr as compared to 0.04 μm NO_3^--N/g soil/hr in the nonplanted treatment. These results support the observed highest NO_3^- concentrations in surficial soils of the planted treatment. The greater depth distribution of nitrification in planted soils follows the depth distribution of root biomass (Table 3) further suggesting the involvement of roots in supplying oxygen to lower depths in the soil.

Table 5. Depth distribution of rates of potential nitrification in planted and nonplanted soils at day 40 of experiment 1.

Treatment	Depth (cm)	μmoles NO_3^- $g \cdot hour^{-1}$
Nonplanted	0- 3	0.040 (0.021)*
	12-15	---**
	21-24	---**
Planted	0- 3	0.167 (0.084)
	12-15	0.041 (0.008)
	21-24	0.004 (0.006)

*Mean followed by standard deviation

**Not detectable

Denitrification potential: Denitrification potential was examined by measuring the rate of nitrous oxide (N_2O) consumption. This assay of denitrification potential has been previously used by Garcia and Tiedje (1982) in paddy soils and Ormeland et al. (1984) in marine intertidal

sediments. It is a useful method for measuring the activity of nitrous oxide reductase, and therefore serves as a tool for assessing denitrification potential.

Nitrous oxide consumption was measured during the tillering, heading and flowering and mature stage of growth which respectively corresponded to 20, 40, and 70 days after transplanting. No significant difference was noted between the activities (Table 6) of nitrous oxide consumption at any of the three growth periods. Differences were however observed between activities in surface (0-2 cm) profiles vs those below the 10-12 cm profile. No significant difference was observed between the planted and nonplanted treatment.

These results were somewhat unexpected since nitrate decreased in concentration (Figure 5) after time of transplanting and was not detectable in soils below 14 cm any time during the treatment period. There also was a significantly lower concentration in surface soils of the nonplanted treatment at all stages during the treatment period. This is in marked contrast to the observed, significant effect of root biomass on the rates of denitrification in agricultural soils by Smith and Tiedje 1979.

Changes in concentrations and distributions of volatile fatty acids: The concentration of volatile fatty acids (VFA's) in planted and nonplanted soil over the treatment period is illustrated in Figures 7. Concentrations of VFA's increased gradually after dry soil flooding and reached a maximum concentration at 30 days after rice transplanting (or 44 days after dry soil flooding). The VFA concentration declined rapidly after this period in planted soils but maintained the same

Table 6. Depth distribution of rates of potential denitrification (ng N_2O-N/g·hr^{-1}) at days 20, 40 and 70 of experiment 1.

Treatment	Depth (cm)	Tillering Stage (20 days)[1]	Heading and Flowering Stage (40 days)	Maturing Stage (70 days)
Planted	-2	0.337 (0.047)	0.382 (0.021)	0.410 (0.033)
	-6	0.319 (0.042)	0.342 (0.039)	0.334 (0.025)
	-10	0.270 (0.039)	0.300 (0.054)	0.325 (0.058)
	-14	0.253 (0.036)	0.281 (0.053)	0.281 (0.052)
	-18	0.227 (0.055)	0.253 (0.045)	0.204 (0.024)
	-22	0.174 (0.066)	0.198 (0.035)	0.207 (0.021)
Non-planted	-2	0.299 (0.013)	0.353 (0.053)	0.375
	-6	0.264 (0.017)	0.315 (0.055)	0.353
	-10	0.238 (0.027)	0.253 (0.081)	0.308
	-14	0.300 (0.010)	0.219 (0.050)	0.251
	-18	0.184 (0.046)	0.240 (0.062)	0.189
	-22	0.154 (0.027)	0.221 (0.016)	0.200

[1] Days after transplanting

[2] Standard deviation

[3] Only one measurement

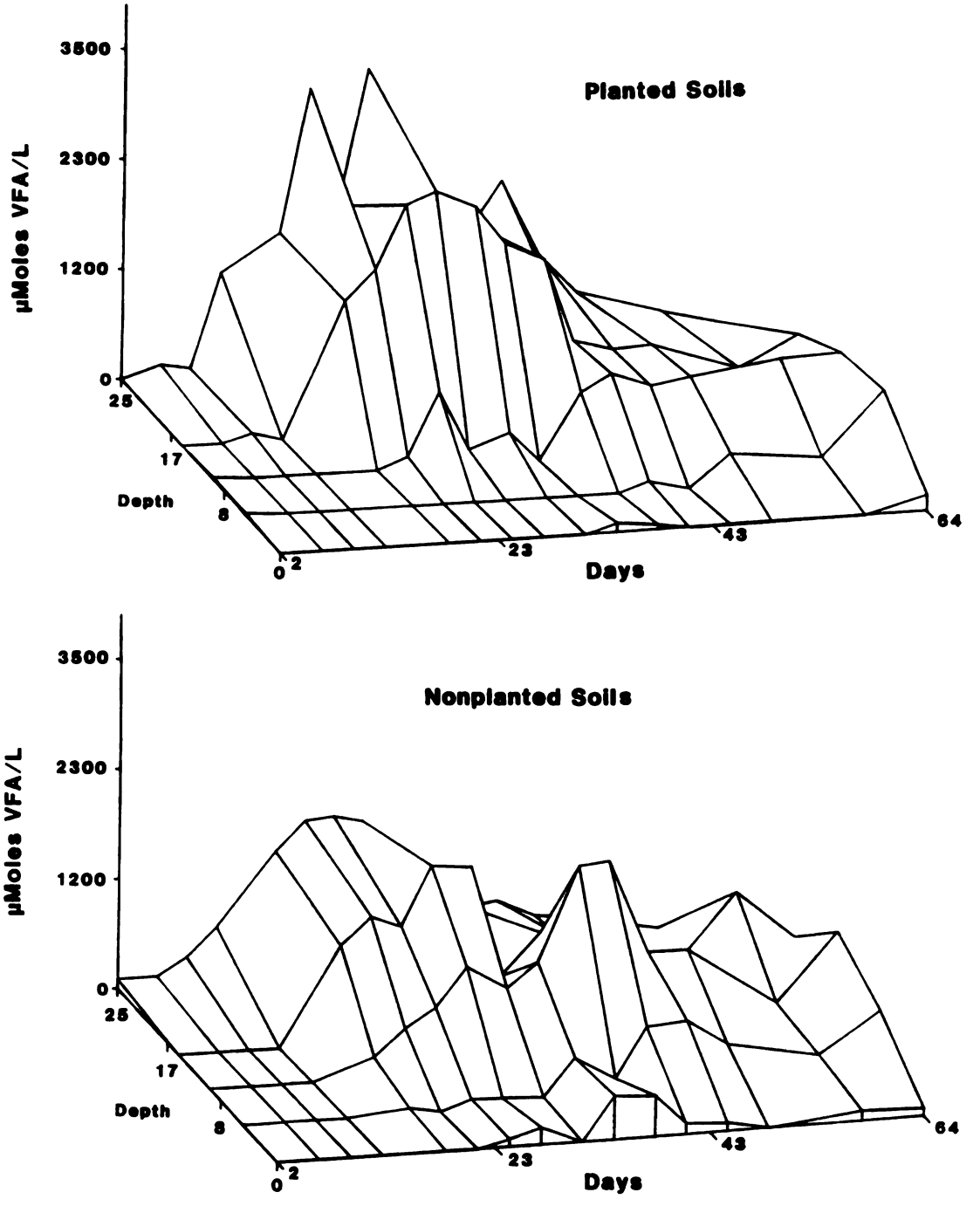

Figure 7. Depth-time distribution of volatile fatty acids (VFA's) in planted and nonplanted soils at 0, 23, 43 and 64 days of the experiment.

qualitative distribution of VFA's during the treatment period. A more gradual decline was observed in the nonplanted soils. Spatial and temporal distribution of VFA's during the treatment period in planted soils is illustrated in Figure 7. Concentrations were consistently higher in the deeper portion of the soil and low to undetectable in surface soils. The same general pattern of VFA distribution was observed in both planted and nonplanted soils. However, the nonplanted soils appeared to have a higher VFA concentration, in the surface soils, and the planted soils had higher concentrations in deeper soils and during the first 60 days after transplanting. It is possible that these increased concentrations are due to increased fermentation of dissolved organic compounds derived from the rooting zone (Chandrasekaran and Yoshida 1973). The vertical distribution of VFA's was inversely related to soil profiles of NO_3^- (Figure 5) and SO_4^{2-} (Figure 8). Lovley and Klug (1986) demonstrated maintenance of low concentration of VFA's in lake sediments when SO_4^{2-} concentration was above 30 μM. Presumably processes of denitrification and sulfate reduction utilize these VFA's as carbon sources and control their concentrations in surface paddy zone. This is also supported, especially in the top 10 cm of soil, by the higher respiratory index (RI) (Figure 12) in nonplanted soil.

Rates of Sulfate Reduction

Sulfate is normally reduced after the depletion of nitrate and other more energetically favorable reactions in anerobic paddy soils (Connel and Patrick 1968, 1969, Ponnamperuma 1972). Sulfate is reduced to H_2S which is toxic to rice at a concentration of approximately 0.07 ppm (Mitsui et al. 1951; Freney et al. 1982). However, H_2S seldom

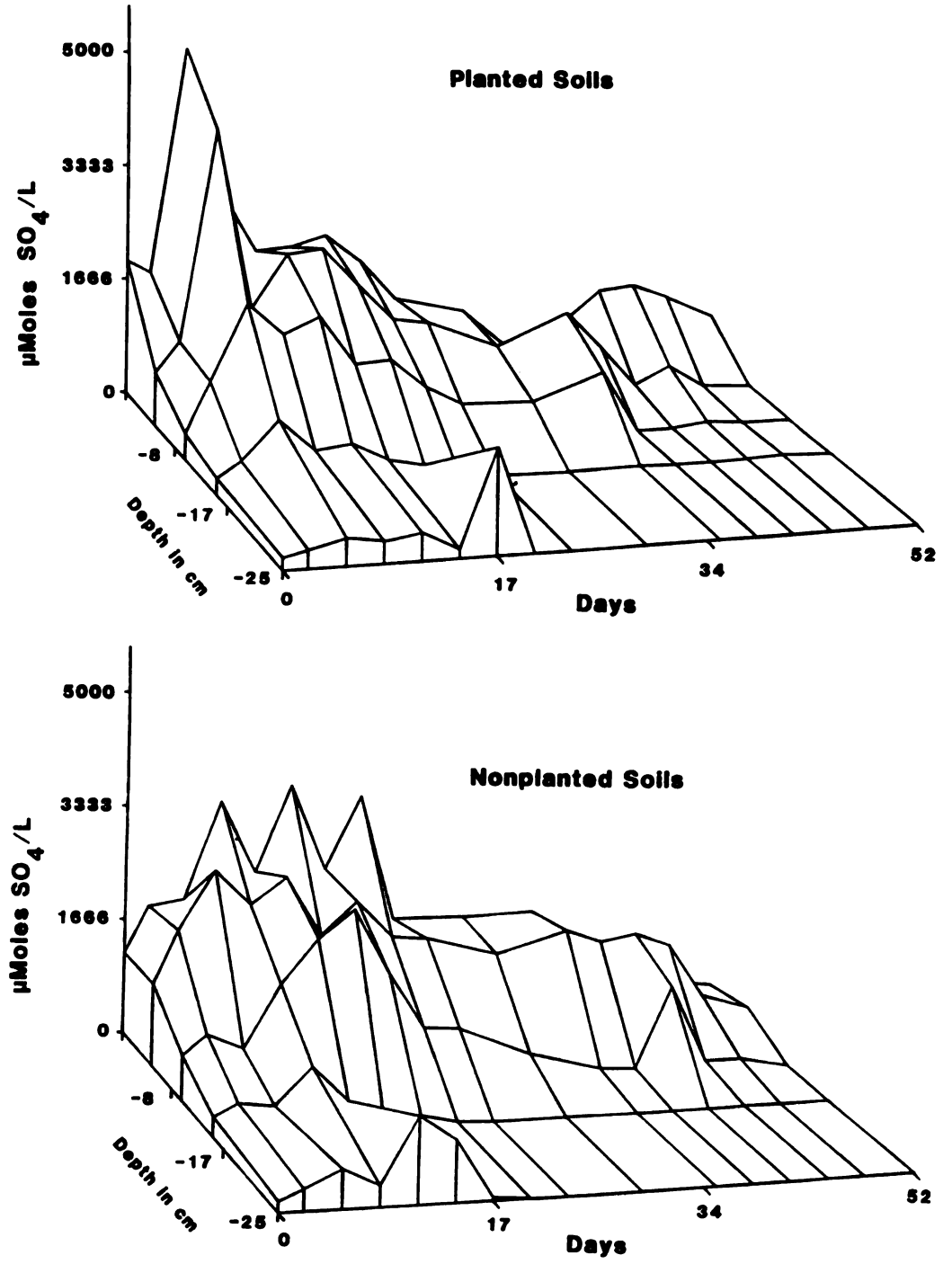

Figure 8. Depth-time distribution of sulfate in planted and nonplanted soils at 0, 17, 34 and 52 days of the experiment.

accumulates at toxic concentrations in most paddy soils, since H_2S is either immediately precipitated as metallic sulfides, chiefly FeS, or is oxidized to sulfate or elemental sulfur in the rice rhizosphere by chemosynthetic microorganisms. Sulfide toxicity can occur in acid i.e. high sulfate soils especially soils low in organic matter and in highly degraded soils such as Akiochi soils of Japan where ferrous iron content is nearly one fifth of normal paddy soils (Shiga and Suzuki 1964).

Distribution of sulfate in pore water: Distribution of sulfate at different soil depths during the treatment period in planted and nonplanted soils is illustrated in Figure 8, respectively. Concentrations of sulfate were highest at the beginning of the treatment period and gradually declined during the remainder of the period. The depletion in sulfate in the soil profile was from the lower portion of the profile upward over time of the experiment.

The depletion of $SO_4^=$ in both of the treatments was similar, however, a general trend toward higher concentrations at 8 cm was noted in the planted treatment. Since both the rice plants and microorganisms were consuming $SO_4^=$ it would follow that considerable reoxidation of reduced sulfur compounds must be occurring in the planted treatment in order to account for the similar $SO_4^=$ profiles in both treatments. This is also supported by the higher sulfate reduction rates observed in the planted treatments (Table 7). This observation suggests increased oxidation of reduced inorganic compounds in the rooting zone of the planted treatment. The rate of sulfate depletion was lower than that of nitrate, supporting the previously discussed spatial and temporal consumption of the most energetically favorable electron acceptor.

Table 7. Depth distribution of rates of sulfate reduction (nmole/g·h^{-1}) at days 20 and 70 of experiment 1.

Treatments	Depth (cm)	Tillering Stage (20 days)*	Maturing Stage (70 days)*
Planted Soil	4	4.7 ± 1.16	8.6 ± 0.91
	8	3.8 ± 0.47	5.9 ± 1.34
	12	2.4 ± 0.81	2.7 ± 0.14
	16	2.5 ± 0.74	2.5 ± 0.49
	20	2.6 ± 1.69	1.9 ± 0.78
	24	1.1 ± 0.79	0.5 ± 0.42
Nonplanted Soil	4	4.9 ± 0.21	4.9 ± 0.42
	8	4.5 ± 1.13	3.7 ± 0.91
	12	1.9 ± 0.18	3.2 ± 1.13
	16	1.5 ± 0.00	2.5 ± 0.21
	20	1.3 ± 1.06	1.8 ± 0.48
	24	0.5 ± 0.28	0.5 ± 0.39

*Days after transplanting

Rates of sulfate reduction were assayed by measuring the depletion of sulfate in pore waters of incubated soils sampled at 20 and 80 days after transplanting. Soils sampled at 80 days were amended with Na_2SO_4 to a final concentration of 1 mm since sulfate was markedly depleted in samples taken below 8 cm (Figure 8). Ingvorsen et al. 1981 found no affect of added sulfate on rates of sulfate reduction if sulfate was above 30 µm. In all treatments the concentration of sulfate was above this value. In both treatments and sampling dates, rates of sulfate reduction decreased with depth which corresponds to concentration profile of $SO_4^=$ (Figure 8). Significant differences were observed between the rates in samples taken at the depth of 0-8 cm at 20 and 80 days in the planted treatments. This is also the zone of the greatest root biomass (Table 4) and the higher rate observed at 80 days is likely due to the absence of NO_3 (Figure 5) and the potentially higher concentration of root exudates and microbial metabolites in this zone in the later stages of growth. This is partially supported by the increasing concentration of VFA's in soils at 64 days after transplanting (Figure 7).

The finding of no significant difference in rates at any depth between the 20 and 80 day samples in the nonplanted treatment supports the concept that plant carbon stimulated the rates of sulfate reduction in soils of the planted treatment. The lack of any significant difference in rates observed in both treatments at 20 days suggests that the high NO_3^- levels observed (Figure 5) inhibits the rates in both of the treatments regardless of the influence of plant carbon on sediment microbial processes.

Methane Production and Emission from Planted and Nonplanted Soils

Changes in methane concentration: Methane concentrations in soils of planted and nonplanted soils sampled at various depths and times during the treatment period are illustrated in Figure 9. Methane concentration increased markedly during the first 20 days after flooding especially in the deeper zones (16-24 cm). Methane concentrations were generally higher in nonplanted soils at all depths and all times of sampling. Differences in concentration could be explained by differences in methane production (see below) or by differences in oxidation especially in surface soils 0-4 cm and at all depths in planted soils.

Root biomass was the highest in the 0-8 cm depth (Table 3), however, root biomass was observed over the entire soil profile. Oxygen diffusion from roots may be responsible for higher rates of methane oxidation throughout the rooting zone, as compared to the nonplanted soils. De Bont et al. (1978), using acetylene as a competitive inhibitor of methane oxidation, demonstrated in situ oxidation of methane in flooded soils. Analyses of methane in the atmosphere above the pots of flooded soils showed significant increase in the amount of methane evolved, but only in the presence of acetylene. In the absence of acetylene, methane was evidently oxidized at the surface-oxidized layer. Holzapfel-Pschorn et al. (1985) reported that 80% of the methane produced in planted paddy soils was oxidized in the rice rhizosphere.

Another explanation for the lower methane concentration in planted soils is the potential for methane to diffuse into the roots and be transported through the lacunae of rice plants to the atmosphere above the soil and water environment. Transport such as this has been

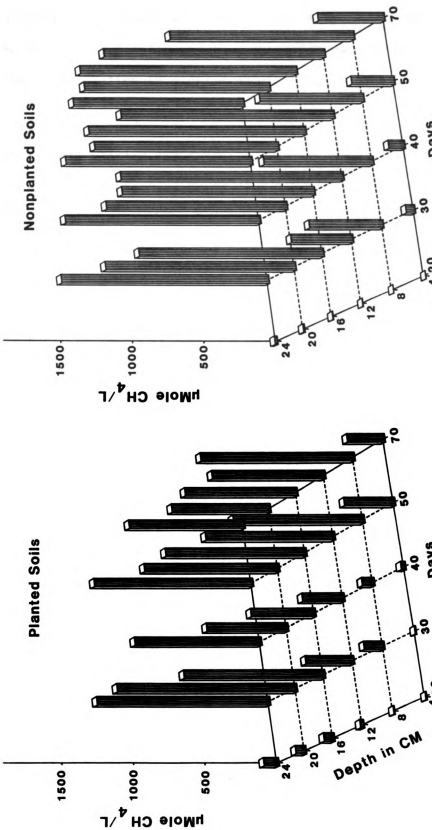

Figure 9. Depth-time distribution of methane in planted and nonplanted soils at 20, 30, 40, 50 and 70 days of the experiment.

reported for other aquatic plants (Dacey and Klug 1979, Sebacher et al. 1985) and in rice (Seiler et al. 1984). The relationship between these various mechanisms is complex and both appear to have a major affect on the methane concentration in planted paddy soils.

Rates of methane production: Rates of methane production increased with depth in both planted and nonplanted soils (Figure 10). Highest production rates were observed at 40 days after flooding, followed by decreased production over the remainder of the treatment period. The lowest rates of production were observed in samples obtained at 20 days and in the 0-4 cm depth zones. Nitrate (Figure 5) and $SO_4^=$ (Figure 6) was observed at varying depths at 20-40 days and in the 0-8 cm zones during the first 60 days. Nitrate or $SO_4^=$ would preferentially be used as electron acceptors in zones where these ions are observed. The distribution pattern of the rates of methane production is similar to that shown for methane concentration, and exhibits an inverse relationship with pore water nitrate and sulfate concentrations, especially during the early period of rice growth (0-20 days). Koyama (1963) indicated that Eh decreased to less than -200 MV before methanogenesis was observed, and the presence of NO_3^- and SO_4^{2-} inhibited the activities of methanogens (Koyama, 1963).

Mutual exclusion of methane and sulfate in sediment interstitial water has been reported (Martens and Berner 1974, Mah et al. 1977). Cappenberg (1974) suggested that the negative interactions between sulfate-reducing and methane-producing bacteria in lake sediments was due to the sulfide, produced by sulfate reduction, which inhibited methane production. Martens and Berner (1974) suggested that methane

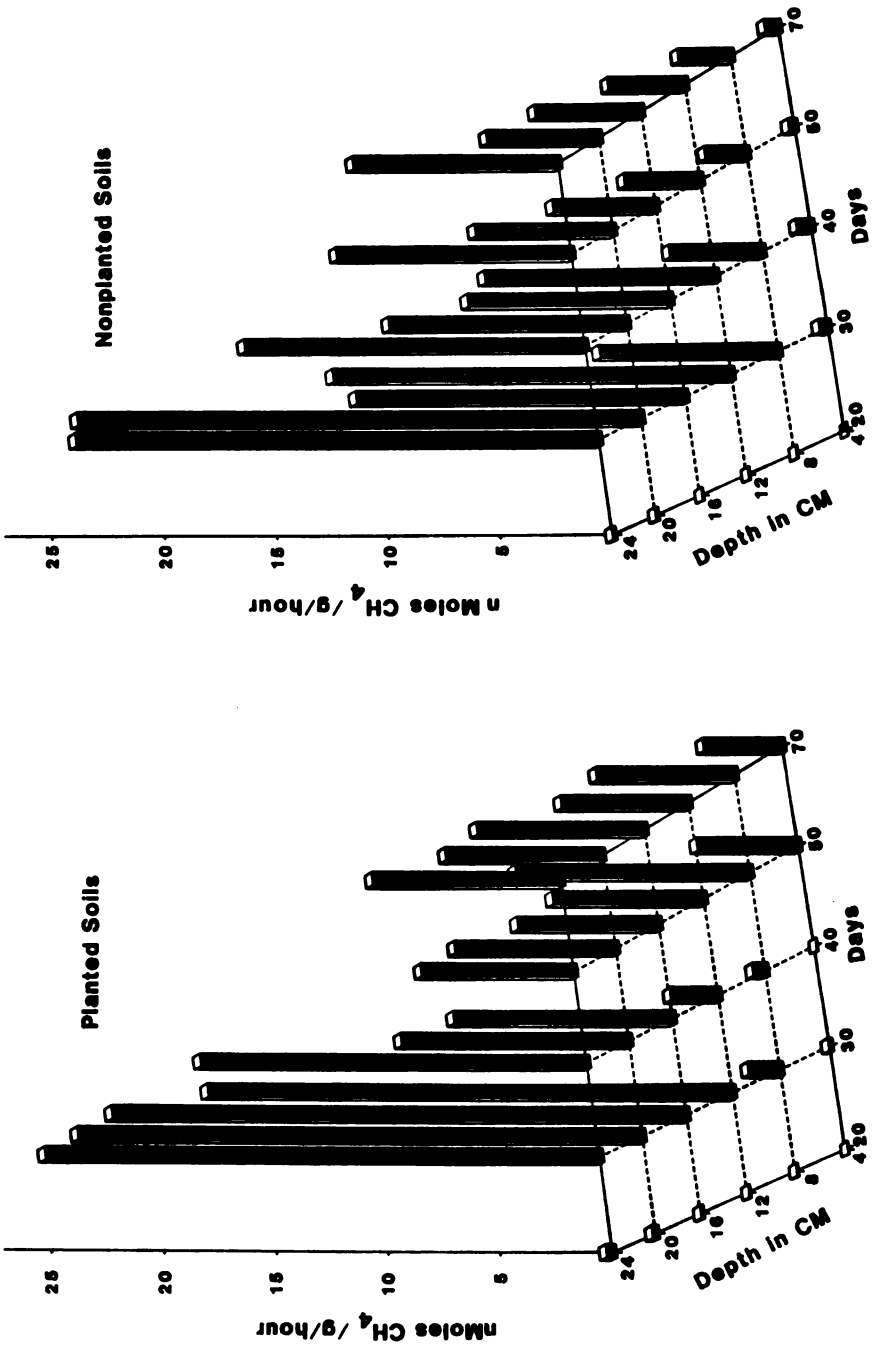

Figure 10. Depth-time distribution of the production of methane in planted and nonplanted soils at 20, 30, 40, 50 and 70 days of the experiment.

production and sulfate reduction occur simultaneously, but that methane is consumed by sulfate-reducing bacteria in sulfate-containing sediments. However, Lovley and Klug (1986) demonstrated that at sulfate concentration greater than 30 μM sulfate reducers maintain acetate and/or H_2 concentrations at concentrations too low for methanogens to grow. The relationship of the distribution of methane concentration and sulfate concentrations and indirectly to their respective role in carbon metabolism at various times and depths in soils during this study is illustrated in Figure 11. This profile demonstrates that at sulfate concentrations above 250 μm, methane concentration is maintained below 10 μm. The lower RI in samples examined in soils sampled at 60 days after flooding (Figure 12) further illustrates that anaerobic respiratory metabolism dominates terminal carbon metabolism during this period. Although there was considerable variance in these profiles a significant difference between the two treatments in the intermediate sample depths is observed and a clear trend toward lower values was observed in the planted soil.

The observed increase in methane production at 60-80 days in the surficial 0-12 cm is at a point where the NO_3^- has been depleted throughout the soil profile and sulfate has been markedly reduced. This is also a time when the above and belowground biomass is maximum. During this stage of plant phenology decreased translocation of nutrient and gaseous translocation occurs (Matsuo 1969, Yoshida 1981, Alberda 1953, Okajima 1964). Therefore, it would be expected that methane concentration would increase since less methane would be oxidized and/or translocated through the plant to the atmosphere. The combination of these processes would result in an increase in methane concentration.

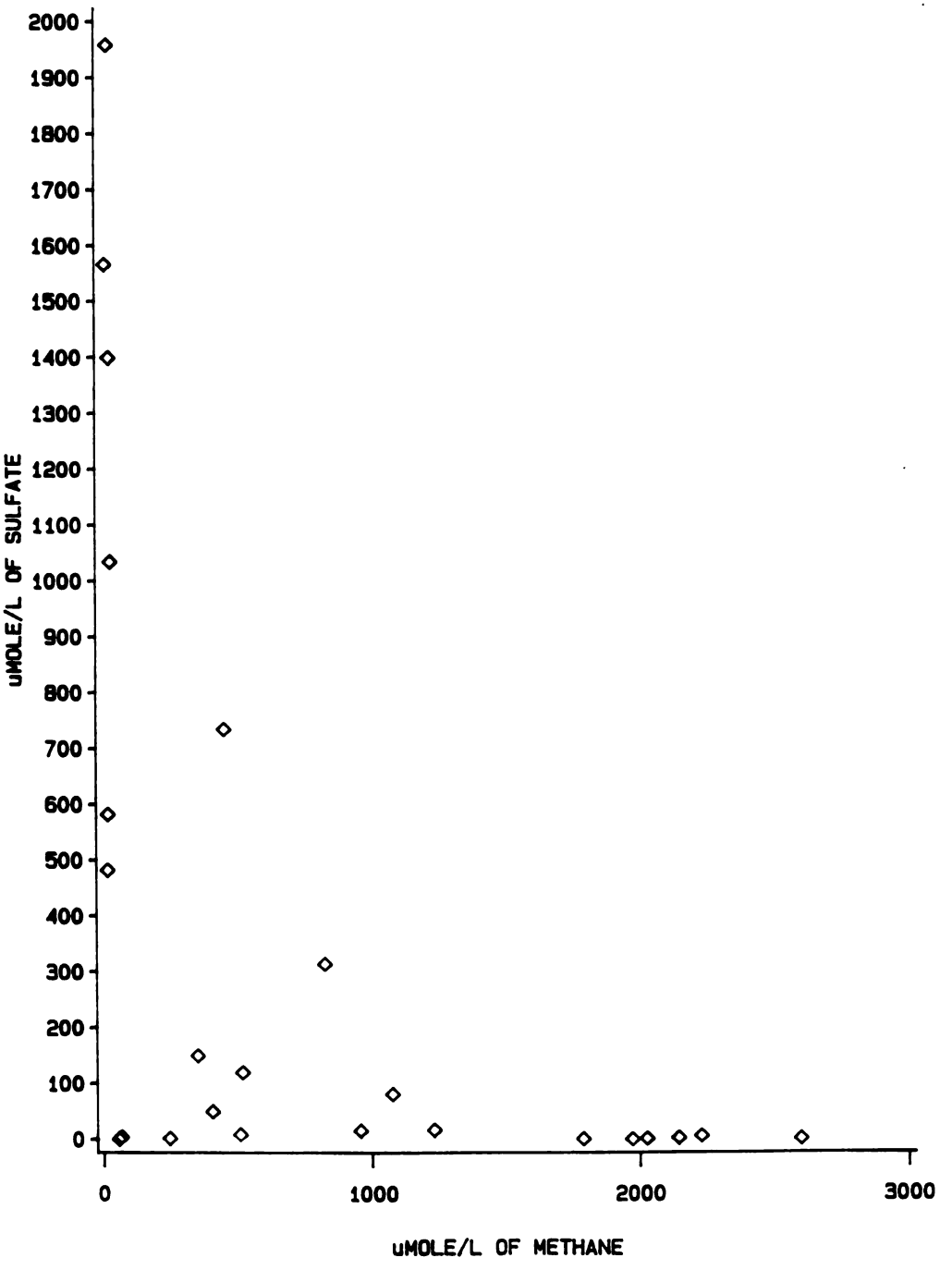

Figure 11. Relationship between the mean concentrations of sulfate and methane in pore waters of planted soils.

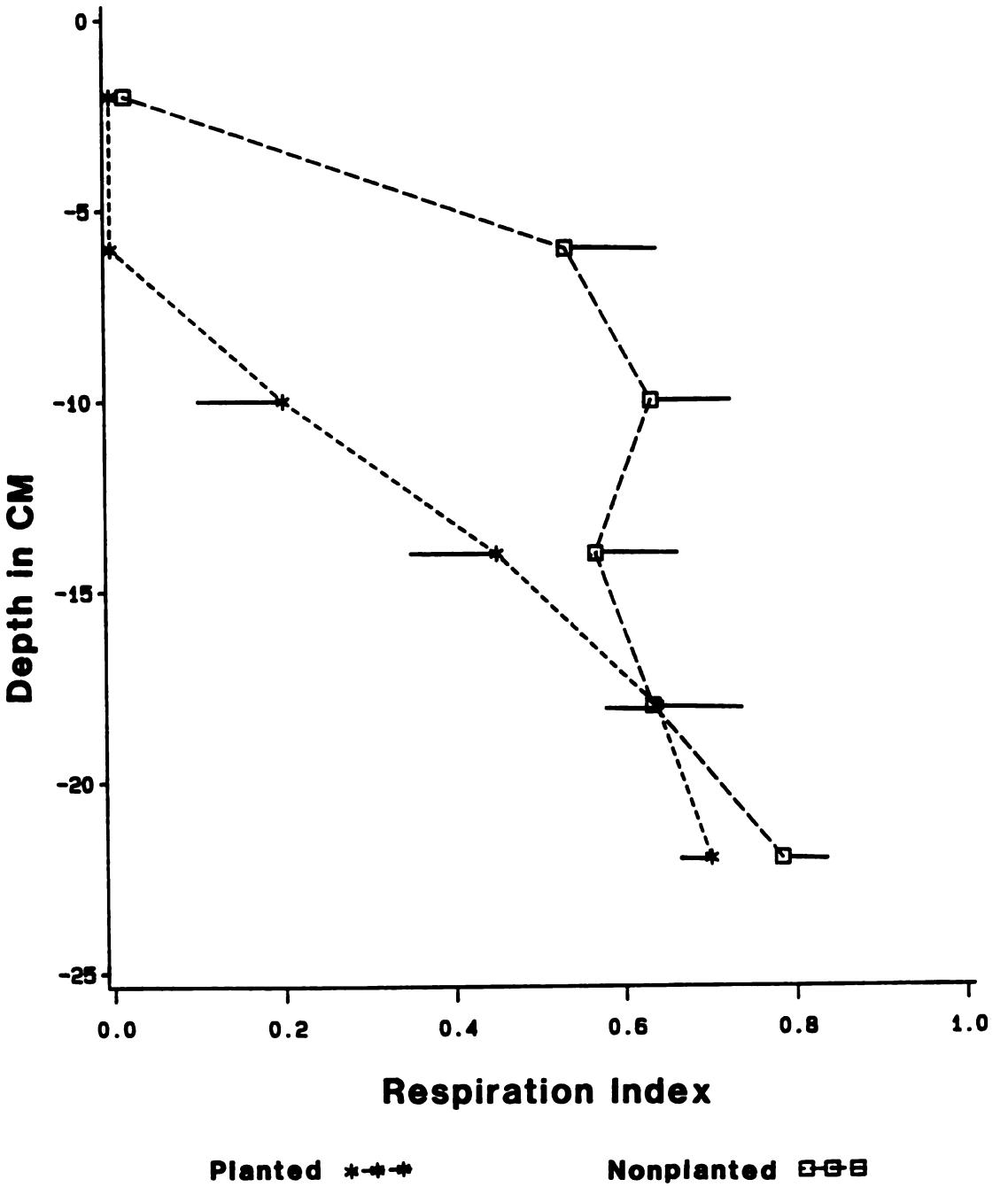

Figure 12. Depth distribution of the respiration index [$^{14}CH_4/C(^{14}CH_4+^{14}CO_2)$] in planted and nonplanted soils at 60 days of the experiment.

Since the reported values are gross and not net rates of methane production, it is not possible at this time to determine if the observed increases are due to increased production or lower rates of oxidation. In future studies it will be necessary to examine both methane oxidation and production rates to obtain an actual net production value. This is very important if one is to be able to relate net rates of methane production in the rhizosphere to concentrations of methane transported to the atmosphere.

A similar increase in production did not occur in the nonplanted treatment. The concentrations of both NO_3^- and $SO_4^=$ were also low in the nonplanted treatment during this same time period. Therefore, the general decline in methane production rates in nonplanted soils, especially in the top 12 cm, may be due to organic matter depletion since these were previously nonplanted soils.

Methane Emission

Static chamber methods were used for all emission studies. Cicerone and Shetter (1981) and Seiler et al. (1984) previously discussed the problems associated with this approach. In summary, the use of static chambers: (1) establishes an artificial environment of CH_4, CO_2, and H_2O vapor and elevated temperatures; (2) blockage of air movement which could influence flux; (3) possible alteration of flux due to lowered eddy currents in the water column.

In order to minimize these problems flux measurements were kept short to minimize high accumulations of CH_4 and water vapor build up in the chambers. Linear increases in the methane mixing ratios were observed in 80 minutes, and rates were comparable using both the Saran

and glass sampling chambers. Sampling periods were always kept to an intervals which assured less than a 10°C increase in temperature.

Rates of methane emission from planted and nonplanted soils and between rice plants from experiment one (never planted soils) are illustrated in Figure 13. Emission of methane was initially observed 20 days after transplanting. This coincided with the onset of methane production (Figure 10) in these soils. Emission from both planted and nonplanted soils increased, however were significantly different (5% level) at nearly all sampling points. Rates of emission from planted soils ranged from 0.73 (0.13) to 15.69 (4.4) mg $CH_4/m^2/hr$, with the highest rates of emission observed during the heading and flowering stage, and the lowest during the tillering and maturing stage of growth. This temporal pattern and total rate of emission is similar to that reported by Holzapfel-Pschorn et al. (1986) for paddy fields in Vercelli, Italy. The temporal pattern is also similar to that reported by Seiler et al. 1984 from paddy soils in Audalusia, Spain, however, their reported mean rate of emission was 2-fold lower. Comparison of rates between sites is difficult without knowing the rates of production, actual methane concentration in the soil pore water and total soil volume measured at the time rates of emission are measured.

Both Seiler et al. (1984) and Holzapfel-Pschorn et al. (1986) reported significant diurnal differences in methane emission during their studies. Seiler et al. (1984) demonstrated that the diurnal response was not dependent on light and concluded that the diurnal differences were due to differences in soil temperature. Shifts in soil temperature ranged up to 6°C over a diurnal cycle in their studies. Koyama (1963) reported on the significant influence of temperature on

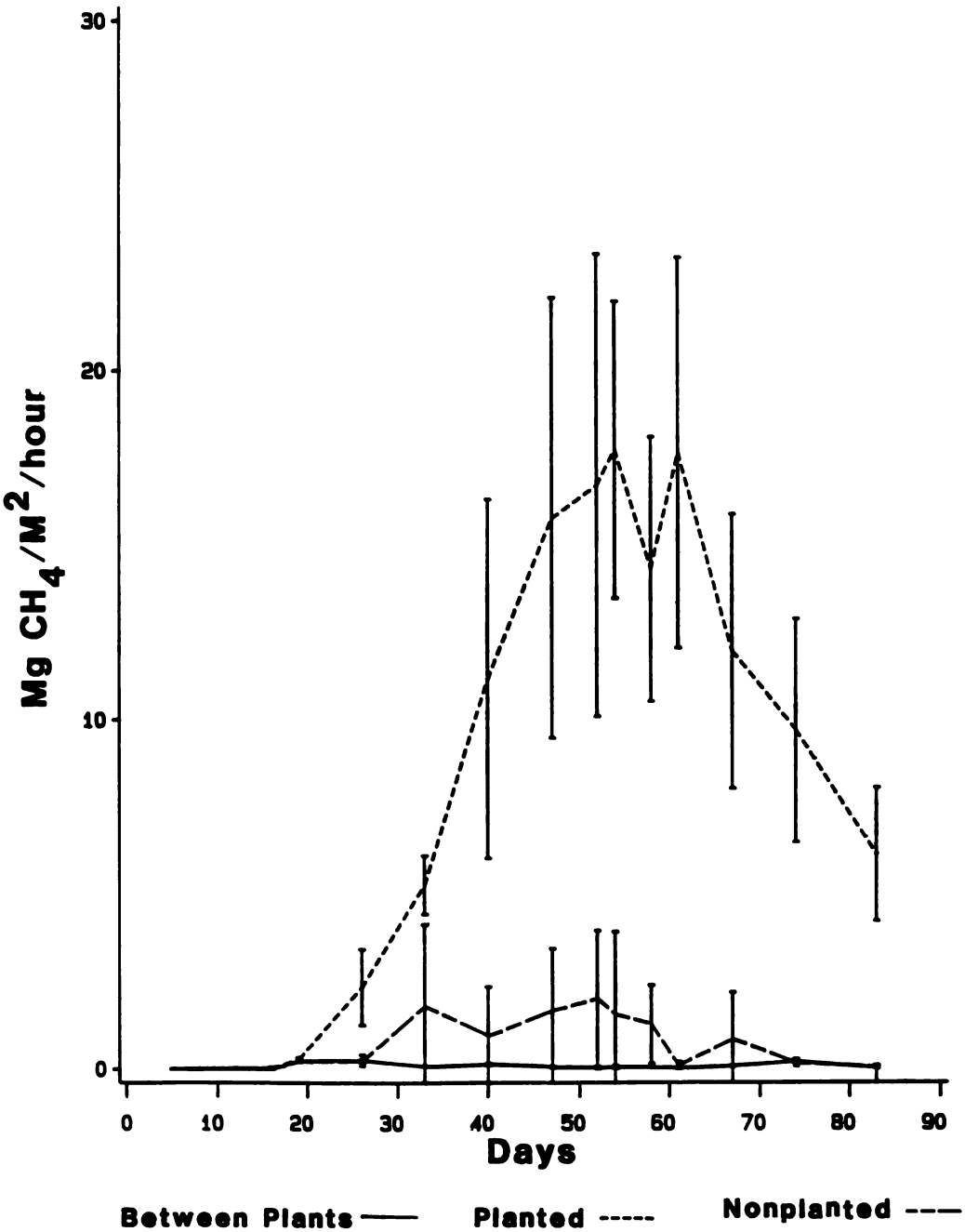

Figure 13. Methane emission from planted, nonplanted soils and between plants in the planted treatment at 10 day intervals during experiment 1.

methane production, which would affect subsequent rates of emission.

No significant diurnal differences in rates of emission were observed in our study, however changes in soil temperature would be expected to be less, since air temperatures only fluctuated 5° during the course of the study. This fluctuation would have had little effect on soil temperature, in any of the treatments. Cicerone and Shetter (1981) also reported small diurnal differences in the rates of emission of methane in paddy soils in Davis, California. They did not record emission during the whole growing season, however they had a maximum diurnal air temperature of 10°C, which may have had little affect on soil temperature.

Little fluctuation in rates of emission from between plants was observed during the growth periods. The mean rate reported during the growth period was 0.10 (0.02) mg $CH_4/m^2/hr$. Holzapfel-Pschorn et al. 1986 reported rates of 1.7 (2.2) mg $CH_4/m^2/hr$ in paddy soils in Vercelli, Italy, and concluded that the majority of the gas escaping between plants was through ebullition.

Few bubbles were noted in our study and differences between the two is most likely due to differences in methane production in the two studies. Our study was conducted in never planted soils while the Holzapfel-Pschorn et al. (1986) study was conducted on paddy soils receiving rice straw. Increased organic matter content of the soil would positively affect rates of methane production, and increase rates of emission. This will be further discussed in later sections on the effect of added organic matter on rates of methane production and emission.

Emission of methane from nonplanted soils was significantly (5%)

different from that in the planted soils during the periods when maximum emission was occurring. Rates ranged from 0.11 (.04) to 1.45 (0.6) mg $CH_4/m^2/hr$. Although the rates are significantly different, the temporal pattern of emission was quite similar.

Since these were never planted soils which were recently flooded one would expect a slow development in the appearance of methane formation which would coincide with the depletion of inorganic electron acceptors, and appearance of precursors of methanogenesis. The rates and temporal patterns of methane production were very similar (Figure 13), however these are net rates of production which do not account for differences in rates of methane oxidation which are expected to be greater in planted soils due to the influence of the plant in providing oxygen in the soil. The similarities in rates of methane production and the major differences in rates of emission strongly suggest an influence of the plants on rates of emission.

Table 8 illustrates the effects of cutting the plants off above and below the water line on rates of methane emission. In the treatment where the plant is cut off below the water line the rates of emission are similar to those observed in nonplanted soils, while those cut above the water line exhibit rates similar to those of the intact plant. The observed inhibition of CH_4 emission by cutting plants below the water line demonstrates that the plants lacunal structure serves as a major transport mechanism for methane from paddy soils. Approximately 94% of the methane emitted is lost through the plant. Similar ranges were reported in other studies with rice, Cicerone and Shetter (1981), Seiler et al. (1984), and Holzapfel-Pschorn (1986), and in other aquatic plants (e.g. Dacey and Klug 1979).

Table 8. Influence of rice plants on the rates of methane emission at the flowering and heading stage of growth. Data reported as mean (n=6) followed by the standard deviation.

CH_4 Emission Rates $mg/m^2/hr$		
Intact plants	Plants cut above water level	Plants cut below water level
11.7(2.1)	10.9(1.9)	0.43(0.10)

The direct relationship between changes in plant biomass and rates of emission supports the above observed effect of the plant on rates of emission. The decline in rates of emission during the maturing stage of growth (> 70 days) is directly correlated to the observed increase in methane concentration in planted soils (Figure 9). It is also noted that rates of methane production also occur at this time (Figure 10) which, as previously discussed, may be the effect of decreased methane oxidation, due to less oxygen supplied to the soils from actively growing plants. It is also possible that increased root exudation at this time could be responsible for increased fermentation and subsequent methane production.

Arashi and Nitta (1955) reported that roots of rice begin to senesce following the flowering and heading stages of growth. This would most likely lead to decreases in the internal structure of the lacunae (Matsuo, 1969) and transport of methane through the plant. Root biomass was only measured through the flowering and heading stage in our study resulting in the inability to determine if root biomass decreased during

the maturing period of this study.

A summary of the temporal relationship between methane production and emission from planted and nonplanted soils is reported on an areal basis in Figure 14. Highest rates of emission from the planted soils are observed when the production is actually shown to be decreasing. As discussed previously this actually could be an artifact of our inability to assess gross methane production. The percent of the methane production emitted from these soils ranged from 1.3-69% with a mean of 27.0 (22.8)%. The highest percentages reported for the period of maximum emission (50-60 days after transplanting). In the nonplanted treatment the percentages ranged from 0.5-4% with a mean of 3.0 (1.4)%. These results support the previous observations of the contribution of the plant to emission rates from the soil, and emphasizes the importance of this mechanism as a source of atmospheric methane.

Influence of previous cropping: Soils from experiment one were maintained under flooded conditions for 90 days. At this time pore water nitrate and sulfate concentrations were 1-2μm and 3-4μm respectively. Active methanogenesis was noted shortly after time of transplanting as illustrated in Figure 15. The absence of metabolites, e.g. NO_3^-, $SO_4^=$ for anaerobic respiratory processes and the presence of senescent root biomass from the previous crop is thought to be responsible for the observed rates of methane production at the beginning of the experiment. A trend toward higher production in the planted treatment could reflect the influence of root exudates or sloughed new root tissue. In general the temporal pattern for maximum rates of production are advanced approximately 15 days over those

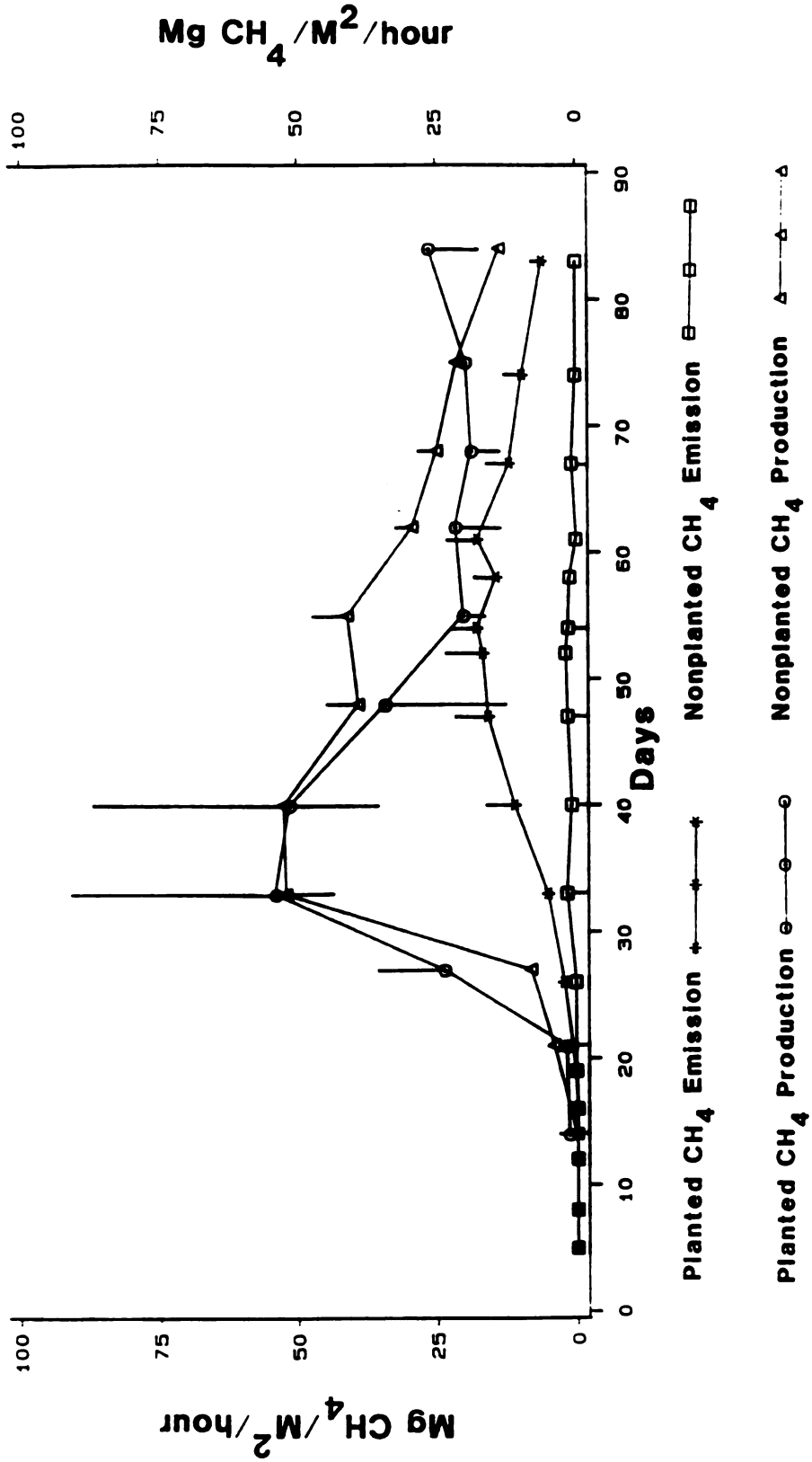

Figure 14. Methane production in and emission from planted and nonplanted soils at 10 day intervals during experiment 1.

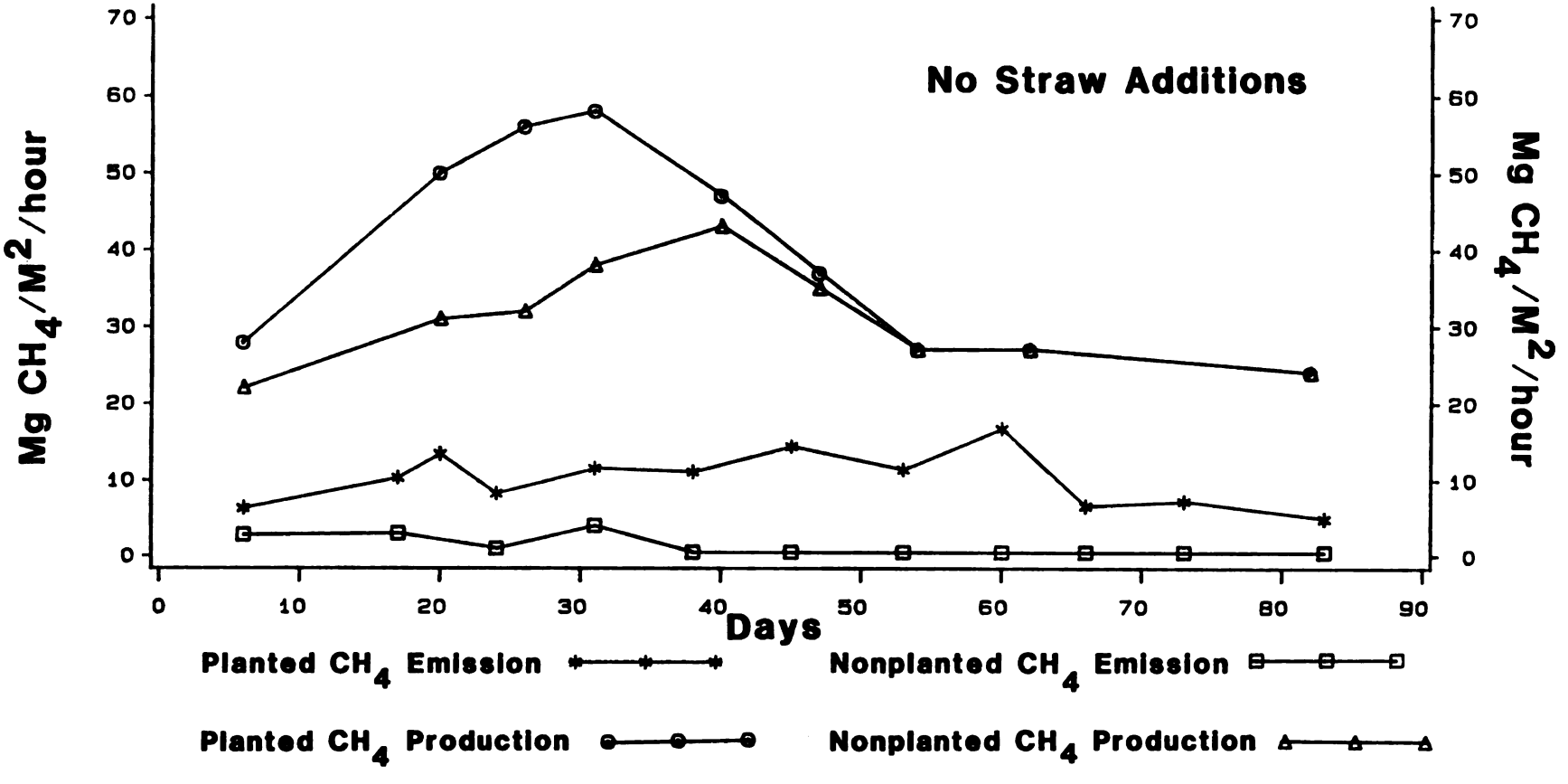

Figure 15. Methane production in and emission from planted and nonplanted previously cropped soils at 10 day intervals during experiment 2.

observed in the never planted soils treatment (Figure 14). Although temporal differences exist, maximum production values for both treatments don't appear to be significant. This is especially true in the planted soils. The range in rates of production in the planted soils were 25-58 mg $CH_4/m^2/hr$ with a mean of 26 mg $CH_4/m^2/hr$. The presence of the plant appears to have a much greater effect on production rates in this treatment as compared to the never planted soils (experiment 1). These differences might reflect the presence of more readily available carbon in the fresh agricultural soils used in experiment 1 which would have been depleted prior to initiation of experiment 2 on these same soils.

Rates of emission were much more constant with time than was reported for experiment 1. The maximum rate (15 mg $CH_4/m^2/hr$) was, however, very similar to that obtained from the never planted treatment. The percent of methane produced which was emitted ranged from 11-55% with a mean of 29.2 (15.1)% in the planted treatment and 0.2-17% with a mean of 5.3 (6.3)% in the nonplanted treatment. These values are quite similar to those reported for the never planted soils. The mean hourly rate of emission from the planted treatment was 9.8 mg $CH_4/m^2/hr$ as compared to 6.3 mg $CH_4/m^2/hr$ for the planted treatment in never planted soils. Therefore, although little differences existed between daily maxima for emission (Figure 14,15) in the two different soils, the longer period of emission in the previously planted soil leads to a higher total emission from these soils.

The influence of previous cropping and straw addition: Rice straw was added to soils previously planted and maintained as described in the

materials and methods.

Temporal patterns of methane emission and production from this treatment are illustrated in Figure 16. The temporal nature of the patterns is quite similar to that observed for previously cropped soils with no straw addition (Figure 15). The major difference is in the elevated rates of production and emission in the straw addition treatment. Differences between the planted and nonplanted treatment are the largest observed for any of the treatments. However, since only one measurement was made at any time point, no significance can be placed on these differences. Rates of production were the highest observed from the three treatments ranging from 26 mg $CH_4/m^2/hr$ to 68 mg $CH_4/m^2/hr$ with a mean of 46 mg $CH_4/m^2/hr$. Rates of emission were similiarly the highest observed ranging from 8.4 mg $CH_4/m^2/hr$ to 24.4 mg $CH_4/m^2/hr$ with a mean of 16.8 mg $CH_4/m^2/hr$. The percentage of the methane produced which was emitted ranged from 20-54% with a mean of 35.7 $\pm$ 10.8.

The overall affect of the addition of previous crop root and straw was an increase in methane production and subsequent emission in both the planted and nonplanted treatments. The percentage of the methane produced which is emitted in both the planted and nonplanted treatment emission was, however, not significantly different. Holzapfel-Pschorn 1986 reported single point percent emitted values which fall within the range of these measurements.

Huang (unpublished data) obtained single time point measurements of methane production, emission, and pore water methane concentrations in two rice growing regions in Taiwan. These values (Table 9) all fall well within the range of our experimental treatments and are closer to those for the previously cropped soils in the experimental treatments.

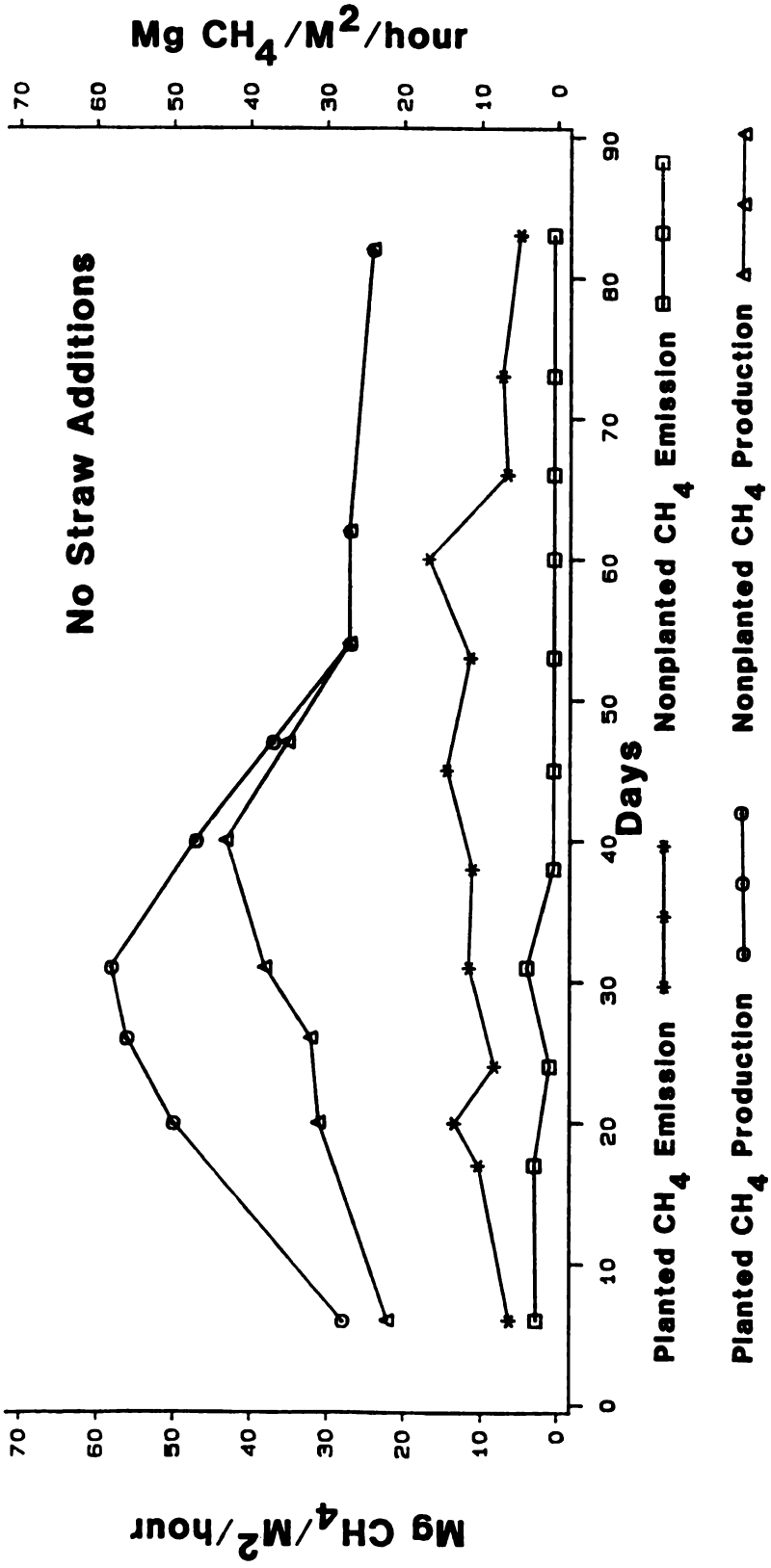

Figure 16. Methane production in and emission from planted and nonplanted previously cropped soils amended with rice straw at 10 day intervals during experiment 2.

Table 9. Methane concentration, rates of methane production, emission and percent of methane production emitted from paddies in the Taichung and Tayei regions of Taiwan in July 1985.

Location	Growth Stage	Field Drainage Condition	Rate of CH_4 emission $mg/m^2/hr$	CH_4 Conc nmol/g	CH_4 Production nmol/g/hr	% CH_4 Production Emitted
Taichung I	Heading & flowering	poorly	13.011	150.3	34.4	38
Tayei I	Heading & flowering	fair-well	6.231	18.3	14.5	43
Taichung II	Maturing	poor-fair	10.881	68.4	29.7	37
Tayei II	Maturing	well	4.630	10	14	33

Interestingly the highest rates of production and emission were in poorly drained soils, which suggests a relationship between drainage rate and rates of methanogenesis. To date no examples of the effect of drainage on rates of methanogenesis exist in the literature. The emission values from the well drained soil are slightly lower than those reported by Cicerone and Shetter 1981 and higher than those reported by Seiler et al. 1984. They are, however, much closer to the range of values reported by both Cicerone and Seiler than the values obtained from the poorly drained soils or the experimental treatments.

Koyama 1963 pointed out the relationship of temperature and methane production. Seiler et al. 1984 emphasized the importance of considering the effects of growing seasonal mean temperature when comparing rates from various sites. It is, however, difficult at this time to separate out the effects of soil temperature, management techniques and the actual rates obtained. These factors must be considered in future studies if meaningful estimates of the effects of these various parameters on methane emissions from paddies around the world.

Effect of nitrogen fertilization: Various combinations of nitrogen fertilizer were added at a rate of 50 Kg N/ha to previously planted soils prior to flooding. Soils were flooded and rice transplanted within one week of flooding. The effects of various combinations is illustrated in Table 10. No significant difference was observed in the production or emission of methane from fertilized treatments over the control values. Although there were no significant differences between treatments the trend was toward higher rates of production and emission, from the NH_4Cl, and control treatments, over those reported for the

Table 10. Effects of N-fertilizer on rates of emission of methane from planted soils.

Nitrogen Source	Amount Used		Rate of Emission	Methane		Sulfate	VFA
	N Kg/ha	$SO_4^{=}$-S Kg/ha	CH_4 $mg/m^2/hr^{-1}$	Conc. µM	Production $mg/m^2/hr^{-1}$	conc. µM	µmoles/pot
$(NH_4)_2SO_4$	50	114	3.012[a**]	96.79[a**]	6.18[a**]	1467[b**]	407
NH_4 Cl	50	0	4.559[a]	179.03[b]	11.38[a]	245[a]	1966
NH_4 Cl plus $FeSO_4$	50	114	3.303[a]	55.85[a]	5.45[a]	2039[b]	100
No N-fertilizer	0	0	3.687[a]	217.08[b]	11.50[a]	280[a]	1581

* Average of 6 measurements from heading and flowering stages of growth.

** In each column means followed by a common letter are not significantly different at 5% level.

nitrogen additions which also included sulfate. As discussed previously, the addition of $SO_4^=$ would be expected to inhibit methane production since sulfate reducing bacteria would be expected to be more competitive than methanogens for common substrates i.e. H_2 and acetate. The lower concentrations of VFA's observed in the sulfate containing treatments supports this concept since the sulfate reducers have a lower Km for VFA's than methanogens.

Cicerone and Shetter (1981) reported significantly higher values of methane emission from plots fertilized with 120 Kg N (as NH_4SO_4) in California rice paddies. Seiler et al. (1984) found no increased methane emission from rice paddies in Spain which were fertilized prior to planting with 160 Kg N/ha (as urea) prior to flooding of the field, and an additional application of 40 Kg N/ha (as NH_4NO_3) which was top-dressed after the tillering stage of rice growth. Seiler et al. 1981 concluded that the relationship of mineral nitrogen to these processes cannot be conclusively determined from these two studies. It would be expected that a complex relationship exists between organic matter additions, temperature, and mineral nitrogen which will affect rates of methane production and emission, from various types of paddy soils.

SUMMARY AND CONCLUSIONS

This study has demonstrated the influence of rice plants on the production of methane in and emission from experimental paddy soils. It was also shown how plants effect the concentrations of the major

inorganic species (NO_3^-, NH_4^+, $SO_4^=$), and volatile fatty acids (VFA's) in the same soils.

The dynamics of the concentrations of both the inorganic species and VFA's was followed by non-destructive sampling of pore water using micro-suction lysimeters, followed by liquid chromatographic analyses. This approach allowed for frequent sampling from the same treatment which was considered as an advantage in establishing the temporal dynamics of the measured parameters.

Initially nitrate concentrations in pore water were high after the flooding of the dry soil. Nitrate concentration in planted treatments was higher, remained longer and even increased in concentration after planting as compared to that in the non-planted treatment. Denitrification potential was slightly higher in planted soil, which follows the trend towards higher NO_3^- concentrations.

Major changes were recorded at depths where the highest root biomass was observed. This suggested the influence of the roots on nitrification and denitrifying activity, through increased oxygen and root exudates in the root zone.

Concentrations of sulfate ($SO_4^=$) were highest at the beginning of the treatment period and gradually declined during the remainder of the period. The depletion in sulfate in the soil profile was from the lower portion of the profiles upward over time of the experiment. The rate of sulfate depletion was slower than that of nitrate, which would correspond to the sequential utilization of inorganic electron acceptors i.e. O_2, NO_3^-, $SO_4^=$.

The concentration VFA's increased gradually after dry soil flooding and reached a maximum concentration at 30 days after rice transplanting

(or 44 days after dry soil flooding), and then declined rapidly, but maintained the same qualitative distribution, during the treatment period. The vertical distribution of VFA's was inversely related to the profile of NO_3^- and $SO_4^=$. This also suggests the sequential use of inorganic electron acceptors, and the accumulation of VFA's following their depletion. The profiles of methane concentrations supported this concept; since the concentration of methane increased, following a decrease in VFA concentration.

Methane concentration increased markedly during the first 20 days after flooding especially in the deeper zones (16 - 24 cm). Methane concentrations were generally higher in non-planted soils at all depths and all times. Differences in concentration might be related to the higher rates of methane oxidation and translocation throughout the rooting zone, as compared to the non-planted soils.

Highest methane production rates were observed at 40 days after flooding, followed by decreased production over the remainder of the treatment period. The lowest rates of production were observed in samples obtained at 20 days and in the 0-4 cm depth zones. Nitrate and sulfate were observed at varying depths at 20-40 days and in the 0-8 cm zones during the first 60 days. Those two ions would preferentially be used as electron acceptors in these zones. A mutual exclusion of methane and sulfate was observed. When the sulfate concentrations were above 250 μm, methane concentration was maintained below 10 μm. Sulfate reducers are known to have higher affinities for acetate and hydrogen and thus can outcompete methanogens for these substrates in the presence of $SO_4^=$. A lower respiration index was also observed at 60 days after flooding which would support this relationship.

Emission of methane was initially observed 20 days after transplanting. Rates of methane emission from planted soils ranged from 0.73 (0.13) to 15.69 (4.4) mg $CH_4/m^2/hr$. with the highest emission rates observed during the heading and flowering stage, and the lowest during the tillering and maturing stage of growth. Emission of methane from non-planted soil was significantly (5%) different from that in the planted soil during the periods when maximum emission was occurring. Rates ranged from 0.11 (0.04) to 1.45 (0.6) mg $CH_4/m^2/hr$.

The percent of the methane production emitted from these soils ranged from 1.3-69% with a mean of 27.0%. The highest percentages reported from the period of maximum emission (50-60 days after transplanting). Most of the emitted methane was observed through the rice plant. These percentages are, however, based on net vs. gross production values, since methane oxidation rates were not examined. Therefore, these percentages may be an overestimate of the actual methane production which is emitted through the plants.

The effect of the addition of previous crop root and straw was an increase in methane production and subsequent emission in both planted and non-planted treatments. No significant difference was observed in the production or emission of methane from fertilized treatments over the control values. However, ammonium sulfate which has $SO_4^=$ residue significantly inhibited the production of methane in the experiment.

The contribution of wetlands to global atmosphere methane concentrations is well documented. These studies have shown that rice plants not only serve as a vehicle for transport of methane from the site of production in the soils to the atmosphere, but stimulate methane production in the soils, presumably due to root exudates. This

stimulation is, however, moderated by the expected increase in methane oxidation in planted treatments due to increased oxygen in these soils supplied through the plant.

Future studies should evaluate net vs. gross methane production rates. They should also be considering manipulations which would enhance oxidation rates i.e. increasing the percolation rate of water through the soils, thereby increasing the potential for increased methane oxidation.

LITERATURE CITED

1. Alberda, T. 1953. Growth and root development of lowland rice and its relation to oxygen supply. Plant and Soil 5:1-10.

2. Ando, T., S. Yoshida and I. Nishyama. 1983. Nature of oxidizing power of rice roots. Plant and Soil 72:57-71.

3. Arashi, K. and H. Nitta. 1955. Studies on the lysigenous intercellular space as the ventilating system in the culm of rice and some other graminaceous plants. Crop Science Soc. Japan Proc. 24:78-81.

4. Armstrong, W. 1969. Rhizosphere oxidation in rice; an analysis of intervarietal difference in oxygen flux from the roots. Physiol. Plant 22:296-303.

5. Armstrong, W. 1978. Root aeration in the wetland condition. Pages 269-297, In: Plant Life in Anaerobic Environments. Hosk, D.E. and R.M.M. Crawford (eds.). Ann Arbor Sci. Publ.

6. Armstrong, W. 1979. Aeration in higher plants. Pages 225-331, In: Advances in Botanical Research. Woodhouse, H.W. (ed.). Vol. 7.

7. Armstrong, W. and T. Webb. 1985. A critical oxygen pressure for root extension in rice. J. Exp. Bot. 36(171):1573-1582.

8. Barber, D.A., M. Ebert and N.T.S. Evens. 1962. The movement of O_2 through barley and rice plants. J. Exp. Bot. 13:397-405.

9. Barker, R. and R.W. Herdt. 1979. Rainfall lowland rice as a research priority - an economist's view. Pages 3-50, In: Rainfed lowland rice. 1978 International Rice Research Conference. IRRI, Los Banos, Philippines.

10. Belser, L.W. and E.L. Mays. 1980. Specific inhibition of nitrite oxidation by chlorate and its use in assessing nitrification in soils and sediments. Appl. Environ. Microbiol. 39:505-510.

11. Bristow, J.M. 1975. The structure and function of roots in aquatic vascular plants. Pages 221-226, In: The Development and Function of Roots. Torrey, J.G. and D.T. Clardson (eds.). Academic Press.

12. Broadbent, F.E. and T. Nakashima. 1970. Nitrogen immobilization in flooded soils. Soil Sci. Soc. Amer. Proc. 34:218-221.

13. Broadbent, F.E. and O.C. Reyes. 1971. Uptake of soil and fertilizer nitrogen by rice in some Philippine soils. Soil Sci. 112:200-205.

14. Cappenberg, Th.E. 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a fresh-water lake. II. Inhibition experiments. Antonie van Leeuwenhoek 40:297-306.

15. Chandrasekaran, S. and T. Yoshida. 1973. Effect of organic acid transformation in submerged soils on growth of rice plants. Soil Sci. Plant Nutr. 19:39-46.

16. Cicerone, R.J. and J.D. Shetter. 1981. Sources of atmospheric methane measurements in rice paddies and a discussion. J. Geophys. Res. 86:7203-7209.

17. Cicerone, R.J., J.D. Shetter and C.C. Delwiche. 1983. Seasonal variation of methane flux from a California rice paddy. J. Geophys. Res. 88:11002-11024.

18. Connel, W.E. and W.H. Patrick, Jr. 1968. Reduction in soil: effects of redox potential and pH. Science 159:86-87.

19. Connel, W.E. and W.H. Patrick, Jr. 1969. Reduction of sulfate to sulfide in waterlogged soil. Soil Sci. Soc. Amer. Proc. 33:711-715.

20. Crutzen, P.J. 1973. A discussion of the chemistry of some minor constituents in the stratosphere and troposphere. Pure Appl. Geophys. 106-108:1385-1399.

21. Dacey, J.W.H. and B.L. Howes. 1984. Water uptake by roots controls water table movement and sediment oxidation in short Spartina marsh. Science 224:487-489.

22. Dacey, J.W.H. and M.J. Klug. 1979. Methane efflux from lake sediments through water lilies. Science 203:1253-1255.

23. DeBont, J.A.M., K.K Lee and D.F. Bouldin. 1978. Bacterial oxidation of methane in rice paddy. Ecol. Bull. (Stockholm) 26:91-96.

24. DeDatta, S.K. 1981. Principles and practices of rice production. John Wiley and Sons. A Wiley-Interscience publication. 618 p.

25. Dommergues, Y.R. and G. Rinaudo. 1979. Factors effecting N_2 fixation in the rice rhizosphere. Pages 241-260, In: Nitrogen and Rice. Edited by the International Rice Research Institute, Los Banos, Philippines.

26. Ehhalt, D.H. 1974. The atmosphere cycle of methane. Tellus 26:58-70.

27. Ehhalt, D.H. and Schmidt. 1978. Sources and sinks of atmospheric methane. Pure Appl. Geophys. 116:452-464.

28. Ehhalt, D.H. 1985. Methane in the global atmosphere. Environment 27:6-33.

29. Food and Agriculture Organization. 1985. Production Yearbook. Vol. 35. Food and Agriculture Organization. Rome.

30. Freney, J.R., V.A. Jacq and J.F. Baldensperger. 1982. The significance of the biological sulfur cycle in rice production. Pages 271-317, In: Microbiology of Tropical Soils and Plant Productivity. Dommergues, Y.R. and H.G. Diem (eds.). Martinus Nijhoff Publ., The Hague.

31. Garcia, J.L. 1973. Reduction de l'oxyde vitreux dans les sols de rizieres dee Senegal: Mesure de l'activite denitrifiante. Soil Biol. Biochem. 6:79-84.

32. Garcia, J.L. and J.M. Tiedje. 1982. Denitrification in rice soils. Pages 187-208, In: Dommergues, Y.R. and H.G. Diem (eds.). Microbiology of Tropical Soils and Plant Productivity. Martinus Nijhoff Publ., The Hague.

33. Greenwood, D.J. and D. Boodman. 1964. Oxygen diffusion and aerobic respiration in soil spheres. J. Sci. Food Agric. 15:579-588.

34. Hayashi, S., K. Asatruma, T. Nagatsuka and C. Furusaka. 1978. Studies on bacteria in paddy soil. Rep. Inst. Agric. Res. Tohoku Univ., 29.

35. Helal, H.M. and D.R. Sauerbeck. 1983. Method to study turnover processes in soil layers of different proximity to roots. Soil Biol. Biochem. 15(2):223-225.

36. Holzapfel-Pschorn, A., R. Conrad and W. Seiler. 1985. Production, oxidation and emission of methane in rice paddies. FEMS Microbiology Ecology 31:343-351.

37. Holzapfel-Pschorn, A., R. Conrad and W. Seiler. 1986. Effects of vegetation on the emission of methane from submerged paddy soil. Plant and Soil 92:223-233.

38. Howes, B.L., J.W.H. Dacey and S.G. Wakeham. 1985. Effects of sampling technique on measurements of pore water constituents in salt marsh sediments. Limnol. Oceanogr. 30(1):221-237.

39. Ingvorsen, K., J.G. Zeikus and T.D. Brock. 1981. Dynamics of bacterial sulfate reduction in a eutrophic lake. Appl. Environ. Microbiol. 42:1029-1036.

40. Inoko, A. 1984. Compost as a source of plant nutrients. In: Organic Matter and Rice. International Rice Research Institute,

Los Banos, Laguna, Philippines.

41. Jørgensen, B.B. 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. Geomicrobiol. J. 1(1):11-27.

42. Joshi, M.M. and J.P. Hollis. 1977. Interaction of Beggiatoa and rice plant: detoxification of hydrogen sulfide in the rice rhizosphere. Science 195:179-180.

43. Kamura, T., Y. Takai and K. Ishikawa. 1963. Microbial reduction mechanism of ferric iron in paddy soils. Soil Sci. Plant Nutr. (Tokyo) 9:171.

44. King, G.M. and M.J. Klug. 1982. Comparative aspects of sulfur mineralization in an eutrophic lake basin. Appl. and Environ. Microbiol. 43:1406-1412.

45. Komatsu, Y., M. Takagi and M. Yamaguchi. 1978. Participation of iron in denitrification in waterlogged soil. Soil Biol. Biochem. 10:21-26.

46. Koroleff, F. 1970. Direct determination of ammonia as indophenol. ICES, Interlab Rep. 3:19-22.

47. Koyama, T. 1963. Gaseous metabolism in lake sediments and paddy soils and the production of atmospheric methane and hydrogen. J. Geophys. Res. 68:3971-3973.

48. Levy, H. 1971. Normal atmosphere: Large radical and formaldehyde concentrations predicted. Science 173:141-143.

49. Lin, C.F. 1982. The recycling of crop residues in Taiwan. Food and Fertilizer Technology Center. ASPAC. Ext. Bull. 77.

50. Lovley, D.R. and M.J. Klug. 1982. Intermediary metabolism of organic matter in the sediments of an eutrophic lake. Appl. and Enviorn. Microbiol. 43:552-560.

51. Lovley, D.R. and M.J. Klug. 1986. Model for the distribution of sulfate reduction and methanogenesis in freshwater sediments. Geochimica et Cosmochimica Acta 50:11-18.

52. Lovelock. J.E. and L. Margulis. 1974. Atmospheric homeostasis by and for the biosphere: The GAIA Hypothesis. Tellus 26:2-9.

53. MacRae, I.C., R.R. Aucajas and S. Salandanan. 1968. The fate of nitrate nitrogen in some tropical soil following submergence. Soil Sci. 105:327.

54. Mah, R.A., D.M. Ward, L. Baresi and T.L. Glass. 1977. Biogenesis of methane. Ann. Rev. Microbiol. 31:309-341.

55. Martens, C.S. and R.A. Berner. 1974. Methane production in the

interstitial waters of sulfate-depleted marine sediments. Science 185:1167-1169.

56. Matsuo, T. 1969. Morphology and Functions of Rice Plants. 6th Ed. Association Agri. Technology, Tokyo, Japan.

57. McConnell, J.C., M.C. McElroy and S.C. Wofsy. 1971. Natural sources of atmospheric CO. Nature 223:187-188.

58. Mitsui, S., S. Aso and K. Kumazawa. 1951. Dynamic studies on the nutrient uptake by crop plants. 1. The nutrient uptake of roots as influenced by hydrogen sulfide. J. Sci. Soil and Manure, Japan 22:324-328.

59. Okajima, H. 1964. Environmental Factors and Nutrient Uptake. International Rice Research Institute 4:63-73.

60. Oremland, R.S., C. Umberger, C.W. Culbertson and R.L. Smith. 1984. Denitrification in San Francisco Bay intertidal sediments. Appl. Environ. Microbiol. 47:1106-1112.

61. Palacpac, A.C. 1982. World rice statistics. International Rice Research Institute, Los Banos, Philippines. 152 pp.

62. Patrick, W.H., Jr. and M.B. Sturgis. 1955. Concentration and movement of oxygen as related to absorption of ammonium and nitrate nitrogen by rice. Soil Sci. Soc. Amer. Proc. 19:59-68.

63. Ponnamperuma, F.N. 1972. The chemistry of submerged soils. Adv. in Agron. 24:29-97.

64. Ponnamperuma, F.N. 1984. Straw as a source of nutrients for wetland rice. Pages 117-136, In: Organic Matter and Rice. International Rice Research Institute, Los Banos, Laguna, Philippines.

65. Rao, D.N. and D.S. Mikkelsen. 1977. Effects of acetic, propionic, and butyric acids n rice seedling growth and nutrition. Plant and Soil 47:323.

66. Rao, D.N. and D.S. Mikkelsen. 1977. Effect of rice straw additions on production of organic acids in flooded soil. Plant and Soil 47:303-311.

67. Rao, D.N. and D.S. Mikkelsen. 1976. Effect of rice straw incorporation on rice plant growth and nutrition. Agron. J. 68(58):752-760.

68. Rao, P.S.C., R.E. Jessup and K.R. Reddy. 1984. Simulation of nitrogen dynamics in flooded soils. Soil Sci. 138(1):54-62.

69. Reddy, K.R. 1982. Nitrogen cycling in a flooded soil ecosystem planted to rice (Oryza sativa L.) Plant and Soil 67:209.

70. Reddy, K.R. and W.H. Patrick, Jr. 1986. Fate of fertilizer nitrogen in rice root zone. Soil Sci. Soc. Amer. J. 50:649-651.

71. Rudd, J.W.H. and R.D. Hamilton. 1979. Methane cycling in Lake 227 in perspective with some components of the carbon and oxygen cycles. Archiv. fur Hydrobiologie ergbenis der Limnologie 12:115-122.

72. Seiler, W. 1984. Contribution of biological processes to the global budget of CH_4 in the atmosphere. Pages 468-477, In: Current Perspectives in Microbial Ecology. Klug, M.J. and C.A. Reddy (eds.). Amer. Soc. Microbiol.

73. Seiler, W. A. Holzapfel-Pschorn, R. Conrad and D. Scharffe. 1984. Methane emission from rice paddies. J. of Atmosph. Chem. 1:241-268.

74. Sethunathan, N., N.R. Rao, T.K. Adhya and R.G. Raghu. 1982. Microbiology of rice soils. Crit. Rev. in Microbiol. 10(2):125-173.

75. Shiga, H. and S. Suzuki. 1964. Studies on the behavior of hydrogen sulfide in waterlogged soils. VII. Bull. Chugoku Agric. Exp. Stn.

76. Smith, C.J. and R.D. Delaune. 1984. Effect of rice plant on nitrification-denitrification loss of nitrogen under greenhouse conditions. Plant and Soil 79:287-290.

77. Smith, P.S. and R.E. Hungate. 1958. Isolation and characterization of Methanobacterium ruminatiun. sp. n. J. of Bacteriol. 75:715-718.

78. Smith, M.S. and J.M. Tiedje. 1979. The effect of roots on soil denitrification. Soil Sci. Soc. Amer. J. 43:951-955.

79. Stolarski, R.S. and R.J. Cicerone. 1974. Stratospheric chloride: possible sink for ozone. Can. J. Chem. 52:1610-1615.

80. Taiwan Agriculture Yearbook. 1985. Dept. of Agric. and Forest, Taiwan Provi. Govern., ROC.

81. Takai, Y. 1970. The mechanism of methane fermentation in flooded paddy soil. Soil Sci. and Plant Nutr. (Tokyo) 16:238-245.

82. Takai, Y. and C. Tezuka. 1971. Sulfate-reducing bacteria in paddy and upland soils. J. Sci. Soil Manure, Japan 42:145-154.

83. Takeda, K. and C. Furusaka. 1975. Studies on the bacteria isolated anaerobically from paddy field soil. III. Production of fatty acids and ammonia by Clostridium species. Soil Sci. Plant Nutr. 21:113-121.

84. Tanaka, A. 1975. Reutilization of nitrogen and energy of the

straw for the rice cultivation (in Japanese). J. Sci. Soil and Manure (Japan) 46:328-332.

85. Tate, R.I. 1979. Effect of flooding on microbial activities in organic soils: carbon metabolism. Soil Sci. 128:267.

86. Tiedje, J.M., A.J. Sexstone, T.B. Parkin, N.P. Revsbech and D.R. Shelton. 1984. Anaerobic processes in soil. Plant and Soil 76:197-212.

87. Trolldenier, G. 1977. Mineral nutrition and reduction processes in the rhizosphere of rice. Plant and Soil 47:193-202.

88. Trolldenier, G. 1988. Visualisation of oxidizing power of rice roots and of possible participation of bacteria in iron deposition. Z. Pflanzenernahr. Bodenk 151:117-121.

89. Wakao, N. and C. Furusaka. 1973. Distribution of sulfate reducing bacteria in paddy field soil. Soil Sci. Plant Nutr. 19:47-56.

90. Wang, W.D., Y.L. Yung, A.A. Lacis, T. Mo and J.E. Hansen. 1967. Greenhouse effects due to man made perturbations of trace gases. Science 194:685-690.

91. Ward, G.S. 1974. Decomposition of roots. Pages 175-211, In: Biology of Plant Litter Decomposition. Dickinson, C.H. and G.J.F. Pugh (eds.). London.

92. Watanabe, I. and C. Furusaka. 1980. Microbial ecology of flooded rice soils. Pages 125-169, In: Advances in Microbial Ecology. Alexander, M. (ed.).

93. Wofsy, S.C., J.C. McConnell and M.B. McElroy. 1972. Atmospheric CH_4, CO_2 and CO. J. Geophys. Res. 77:4477-4493.

94. Yoshida, T. 1975. Microbial metabolism of flooded soils. Pages 83-120, In: Soil Biochem. Paul, E.A. and A.D. McLacen (eds.). Marcel Dekker, New York.

95. Yoshida, S. 1981. Fundamentals of Rice Crop Science. The International Rice Research Institute, Los Banos, Philippines.