INFORMATION TO USERS

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

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

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

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

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

UMI

University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313/761-4700  800/521-0600
A study on methane emission from rice paddy fields and the nonstructural carbohydrates in rice plants

Wang, Yongbing, M.A.

Rice University, 1993
RICE UNIVERSITY

A STUDY ON METHANE EMISSION FROM RICE PADDY FIELDS AND THE NONSTRUCTURAL CARBOHYDRATES IN RICE PLANTS

by

YONGBING WANG

A THESIS SUBMITTED IN
PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE
MASTER OF ARTS

APPROVED, THESIS COMMITTEE:

Ronald L. Sass, Advisor and Professor of Ecology and Evolutionary Biology

Frank M. Fisher, Advisor and Professor of Ecology and Evolutionary Biology

John B Anderson, Professor of Geology and Geophysics

Fred T Turner, Adjunct Professor of Ecology and Evolutionary Biology

Stanley C. Tyler, Senior Scientist of National Center for Atmospheric Research

Houston, Texas
March, 1993
ABSTRACT

A STUDY ON METHANE EMISSION FROM RICE PADDY FIELDS AND THE NONSTRUCTURAL CARBOHYDRATES IN RICE PLANTS

by

YONGBING WANG

A dynamic process of the nonstructural carbohydrate translocation in rice plants is presented based on observation during one growing season. Differing amounts of nonstructural carbohydrates might be lost through rice plants due to the experimental plant manipulation. Relative to the CH4 flux from the area of normal plants, significant increases in methane emission were observed in the area of manipulated plants. It is proposed that due to the experimental plant manipulation, more organic deposition entered the soil through rice plants than that under normal condition and, the lost nonstructural carbohydrate resulted in methane emission through the anaerobic methanogenesis in the rice field.
ACKNOWLEDGEMENTS

I am deeply indebted to Dr. Ronald L. Sass and Dr. Frank M. Fisher, who not only helped me out with their expertise and kindness on all the experiments, but actually in every other aspect, including data analysis and writing. Without their guidance and help, this thesis would not have come into existence. I am also very grateful to Dr. Fred T. Turner, who provided many constructing suggestions on both the experimental design and data interpretation. My special thanks are also expressed to Dr. Stanley C. Tyler, who gave helpful opinions on the draft of this thesis.

Also my special thanks are expressed to my wife, Wei Ding who helped me in many aspects, including experiments. My sincere thanks are expressed to Cylette Chambers, Sandra L. Lewis, Lief Sigren, Yongping Gao, Jennifer King, Amy Spencer, Susanne Chan and Jenny Vinier who helped me a lot with the lab or field work. My sincere thanks should also be given to Mike Jund, Patrick Eason and Donald Robinson who gave me some special help on the field work.

I would also like to express my sincere gratitude to all the people who have given me help and encouragement, in one way or another, during my two years' study at this Department.
# TABLE OF CONTENTS

Abstract...........................................................................................................ii

Acknowledgements.........................................................................................iii

List of Tables....................................................................................................vi

List of Figures....................................................................................................vii

I. Introduction...................................................................................................1

II. Materials and Methods...............................................................................9
  2.1. Field Description....................................................................................9
  2.2. Experiments..........................................................................................10
     2.2.1. Methane Flux Measurement..........................................................10
     2.2.2. Soil Incubation Measurements.......................................................11
     2.2.3. Biomass and Total Nonstructural Carbohydrate
          samples...............................................................................................12
  2.3. Biomass Determination and Nonstructural Carbohydrate
      Detection.................................................................................................14
     2.3.1. Biomass Determination................................................................14
     2.3.2. Nonstructural Carbohydrate Determination.................................15
  2.4. Instruments............................................................................................18

III. Results......................................................................................................19
  3.1. Percentage Nonstructural Carbohydrates in Rice Plant
      Tissues....................................................................................................19
     3.1.1. Root System....................................................................................19
     3.1.2. Stem and Base................................................................................21
     3.1.3. Leaf................................................................................................24
     3.1.4. Panicle............................................................................................26
  3.2. Total Nonstructural Carbohydrate Partitioning....................................29
     3.2.1. Control (R-P-) and Root Cutting (R+P-).......................................29
     3.2.2. Normal Root with Half Panicle Removal (R-P1/2+) and Root
Cutting with Half Panicle Removal (R\(^{+P^{1/2}+}\)) .................................. 31
3.2.3. Normal Roots with Panicle Removal (R\(-P^{+}\)) and Root Cutting and Panicle Removal (R\(+P^{+}\)) ........................................... 33
3.3. Methane Production and Emission ................................................... 35
3.3.1. Production from Soil Incubation ................................................. 35
3.3.2. Seasonal Variation of Methane Emission .................................... 37
3.3.3. Methane Flux from the Cylinder-Collar ..................................... 38

IV. Discussion ......................................................................................... 42
4.1. Nonstructural Carbohydrate Translocation ..................................... 42
4.2. Percentage Nonstructural Carbohydrate in Leaf ............................ 45
4.3. Percentage Nonstructural Carbohydrate in Root ............................ 47
4.4. Possible Loss of Nonstructural Carbohydrates Due to Experimental Plant Manipulation ................................................................. 49
4.5. Seasonality of Methane Flux and Production ................................. 52
4.6. Methane Fluxes of the Different Plant Treatments and a Hypothesis ...................................................................................... 55
4.7. Summary and Conclusions ............................................................... 62

V. References ....................................................................................... 64
LIST OF TABLES

Table 1. TreatmentDesignations.........................................................13
Table 2. P Values for t Test of the Difference Between the Flux
Data from the Collars........................................................................40
Table 3. Daily Average of the Methane Emission Rate During
Last 21 Days of Season.......................................................................41
Table 4. The Confidence Level of the Difference among the
TNC/SBS Ratios (P value for t Test).......................................................52
Table 5. Estimation of TNC Losses in the Treatments............................53
Table 6. Predicted TNC Accounting for the Increase of Methane
Emission...............................................................................................57
Table 7. Regression Analysis Results in Figure 14..................................57
Table 8. Flux from Control Plants Measured With Box-Frame
and Collar-Cylinder.............................................................................61
LIST OF FIGURES

Figure 1  Nonstructural Carbohydrate in Root ........................................................... 20
Figure 2  Nonstructural Carbohydrate in Stem ............................................................ 22
Figure 3  Nonstructural Carbohydrate in Plant Base .................................................. 23
Figure 4  Nonstructural Carbohydrate in Leaf ............................................................ 25
Figure 5  Nonstructural Carbohydrate in Leaf ............................................................ 27
Figure 6  Nonstructural Carbohydrate in Panicle ......................................................... 28
Figure 7  Total Nonstructural Carbohydrate Partition ................................................. 30
Figure 8  Total Nonstructural Carbohydrate Partition ................................................. 32
Figure 9  Total Nonstructural Carbohydrate Partition ................................................. 34
Figure 10 (a) Soil Incubation; (b) 1992 Methane Flux; (c) Biomass ......................... 36
Figure 11 (a) Daily Average Flux from Collars; (b) Flux from Collars .......................... 39
Figure 12 (a) Nonstructural Carbohydrate in Leaf; (b) Soil Temperature and Percentage NC in Root; (c) Soil/Water Temperature .................................................. 46
Figure 13 Total Nonstructural Carbohydrate against Structural Part of the Stem and Plant base ................................................................. 51
Figure 14 "TNC" Loss vs "TNC" Accounting for the Increase of Methane Emission ........... 57
I. INTRODUCTION

The upward trend in atmospheric methane (CH$_4$) is estimated at an annual increase of about 1% (Bouwman, 1990) over the past decade. Information from polar ice cores indicates a fairly constant atmospheric methane concentration for the past 160,000 years (U. S. EPA). An approximately exponential increase has more than doubled the atmospheric concentration of CH$_4$ over the past 300 years. This increase correlates closely with global population growth and suggests that the change in methane concentration is linked to anthropogenic activities. Methane is an important greenhouse trace gas. Anthropogenic emissions of CH$_4$ were responsible for nearly 20% of the radiative forcing effects that occurred in the 1980s. Moreover, each CH$_4$ molecule is 20 to 60 times more radiatively effective (i.e. effective in trapping infrared in the Earth's atmosphere) than a CO$_2$ molecule (Dickinson et al., 1986; Houghton et al., 1991). Because methane is so potent, and also because it has a relatively short atmospheric lifetime (approximately 8-11 years) (Cicerone and Oremland, 1988), reductions in methane emissions should be transformed relatively quickly into reductions in potential global atmospheric warming. Methane, therefore, represents an attractive opportunity for controlling greenhouse-gas-induced global climate change.

Anaerobic bacterial activity in the world wide wetland rice cultivation is considered to be a primary source of anthropogenic methane emissions (Asemann et al., 1989; Bouwman, 1990). Methane emissions from flooded rice fields have been estimated to be between 60-170 Tg CH$_4$/year
(Holzapfel-Pschorn and Seiler, 1986; Schütz, et al., 1989a); which represents 25% of the annual methane emission from all sources. The methane from flooded rice fields is produced microbially, from anaerobic decomposition of organic materials. It is estimated that the world's annual rough rice production must increase from today's 473 million tons to 781 million tons — a 65% increase (1.7%/year) by 2020 as demanded by an increasing world population (IRRI, 1988). Thus the methane emission from flooded rice fields is expected to increase over the next three decades. Because the arable land is highly limited in major rice growing areas, the increasing rice demand could only be achieved by intensifying cropping. It is concluded that a 10 to 15% reduction in total methane emissions would stabilize atmospheric CH₄ concentrations and a 20 to 30% reduction in methane emissions, relative to current level, is technically feasible over the long term (IPCC, 1988).

Production and emission of methane to the atmosphere from agricultural wetlands, rice fields, are the result of a complex array of soil processes involving plant-microbe interactions. Emissions of methane from flooded rice fields are the combined net result of CH₄ production, CH₄ oxidation, and CH₄ transport to the atmosphere. A comprehensive understanding of the complex interaction between methane production and oxidation, as well as of the exchange of methane between rice fields and the atmosphere is a prerequisite for determining potential options on reduction of methane emission rates. However, the current knowledge on methane production, oxidation and emission processes in rice fields is insufficient.
Methane is produced in flooded rice fields by utilization of organic material by methanogenic bacteria. This begins only after anoxic, reduced soil conditions have been established in the paddies. Approximately 60% (Sass et al., 1990) to 80% (Holzapfel-Pschorn et al., 1985) of the produced methane does not reach the atmosphere, as it is oxidized by aerobic methanotrophic bacteria that are present in the oxic surface layer of the submerged paddy soil and in the rhizosphere where oxygen is available around the rice roots. Methane oxidation changes seasonally, from 18% in the early growing season to 91% near the end of the season (Sass et al., 1992). The remaining, non-oxidized methane is transported from the submerged soil to the atmosphere by diffusion through the flood water, by ebullition, and by plant-mediated transport. Transport by diffusion through the paddy water is of minor importance and about 80% of the observed methane transport is through the intercellular space system of the rice plant (Holzapfel-Pschorn et al., 1986). Nouchi, et al. (1990) reported that methane was mostly released from the culm which is an aggregation of leaf sheaths, but not from leaf blade. They found micropores, which are different from stomata, in the abaxial epidermis of the leaf sheath. These micropores were thought to be the sites where methane is released to the atmosphere.

Methane emission is influenced by temperature, water regime, root exudates, organic soil residues, plant physiology and soil physical, chemical and biological properties (Conrad, 1989; Minami, 1990). Various management practices could directly influence these factors and thus affect the methane emission from rice fields. A five-fold increase in CH₄
emission was observed during some days after the application of (NH$_4$)$_2$SO$_4$ at a rate of 140 kg N ha$^{-1}$ yr$^{-1}$ (Cicerone and Shetter, 1981). However, a significant decrease in CH$_4$ emissions was observed when the same type of fertilizer was applied to Italian rice fields (Schütz, et al., 1989a). It has been suggested that re-oxidation of sulfide to sulfate in the rhizosphere may be partly responsible for maintaining a higher redox potential in the reduced layer (Schütz et al., 1989a). Although sulfate and sulfide may be toxic to methanogens (Jakobsen et al., 1981), it is recently suggested that ammonium may be responsible for the inhibition of methanogenesis by applying (NH$_4$)$_2$SO$_4$, because the application of potassium sulfate (K$_2$SO$_4$) does not significantly affect CH$_4$ emission rates (Schütz et al., 1990). That the pre-flood deep application of urea in the soil could reduce CH$_4$ emissions (Schütz et al., 1989a) also suggests that the effect of (NH$_4$)$_2$SO$_4$ on CH$_4$ emissions is due to ammonium ions (NH$_4^+$) since urea is hydrolyzed in soil by the enzyme urease to ammonium carbonate [(NH$_4$)$_2$CO$_3$]. However, the methane emission in the fields with the pre-flood surface application of urea in soil increased with increasing amounts of urea applied (Lindau et al., 1991). Although the processes that mediate the effect of chemical fertilizer application have not been clearly understood, it is possible to find an optimal regime of chemical fertilizer to help reduce CH$_4$ emissions from rice fields.

Water regime is very effective in altering methane emissions from rice paddies. A series of water management experiments (Sass et al., 1992) showed that methane emission rates varied markedly with water regime. A low seasonal total emission (1.2 g m$^{-2}$) is observed in multiple drainage-
reflooding treatment and a high emission (14.9 g m^{-2}) when flooding was postponed until the second half of the growing season. Although the multiple drainage-reflooding management emitted 88% less methane than the normal irrigation, rice grain yield was not reduced. However, the reduced methane emission in this application is achieved at the cost of much more water which is very expensive in areas with low precipitation. Moreover, intermittent drying and wetting of the paddy field may induce nitrification/denitrification process, whereby nitrogen losses are increased and emissions of N_{2}O, a more potent greenhouse gas than CO_{2} and CH_{4}, are higher than under continuous flooding. Therefore, water management as a mitigation practice may only be feasible in areas of lowland irrigated rice with high water availability, but must be coordinated with N-fertilizer applications.

Organic carbon source in inundated rice fields is the most important factor that affects methane emissions. Rice root exudates (including root litter), previous crop residue in the soil, and organic carbon in the flooding water (algae) constitute the main carbon pools available to soil bacteria. The major substrates derived from these pools for direct use by methanogenic bacteria include short chain fatty acids, alcohols, carbon dioxide and hydrogen. These are characterized as readily mineralizable carbon (RMC) (Yagi and Minami, 1990). The organic compounds in the early stage after flooding are mainly the lower volatile organic acids, such as formic, acetic, propionic, and butyric acid (Chandrasekaran et al., 1973). These acids were found to be toxic to rice plants even in soil with neutral pH (Chandrasekaran et al., 1973; Rao et al., 1977). These acids
could also cause root damage and result in more root exudates and thus result in more RMC and subsequent methane emissions. In experiments incorporating rice straw in rice fields, higher methane emission rates in early season and more seasonal methane emission were observed (Schütz et al., 1989; Sass et al., 1991). This suggests that under normal cultivation management, there is a potential for microorganisms in rice fields to produce more methane if crop residues are available. Research in bare plots showed that the carbon pool in the unplanted paddy would be exhausted within approximately two weeks after flooding (Sass et al., 1990). Thus, variations in the total production and emission of methane are ultimately determined by the physiological state of rice plants which may cause additional carbohydrates assimilated from photosynthesis to enter the soil system.

The major part of the starch in rice grains at harvest is the photosynthetic product of the leaves, which is translocated from the leaves to the growing grains through leaf sheath and culm after flowering (Venkateswarlu et al., 1987). The ripening of rice grains can be considered a process of depositing nonstructural carbohydrate from the source, the leaves, to the sink, the developing flower, which is a common model applicable to all the crop plants. The grain yield may be limited by either the size of the sink or the available assimilates in the source. Generally, the photosynthates of rice leaves are transferred to every tissue of the plant for respiration, growth of structural materials, production of biologically active materials (such as enzymes, hormones etc.) and nonstructural carbon storage. A remarkable amount of metabolites, mostly
carbohydrates, organic acids and amino acids, are released through the roots into the soil in the formation of root exudates, which may have its own physiological significance (Sadhu et al., 1969). Some artificial limits on the source or sink of a crop plant may affect the partition of photosynthates in the plant tissues. Circumstantial evidence shows that excluding light from parts of the photosynthetic system of some cereals does not depress grain yield in proportion to the darkened-leaf area (Nosberger et al., 1965). This indicates that the remaining photosynthetic area compensated for this. In tomato, greater photosynthetic translocation from one source-sink unit to the other was observed when the leaves (source) or the truss (sink unit) was removed (Tanaka et al., 1974). Flowering alfalfa plants produced a significant increase in amounts of material in the root exudates over that from the clipped non-flowering plants (Hamlen et al., 1971a, b). This supports the idea that plant age and stage of development have significant influence on the qualitative and quantitative nature of plant root exudates. Shoot clipping research with Western Wheatgrass (Bokhari et al., 1974) showed an increase in the carbohydrate content in the root exudates proportional to the degree of clipping. More carbohydrate was also found in the root exudate under regimes of higher temperature. These suggest that disturbances on the normal source-sink relationship may affect the material exchange between plant and soil and thus affect the rhizosphere organic deposition.

Some evidence suggests that the carbon substrate for methanogenic bacteria in the soil is mainly supplied by the same pool of carbohydrate that is used by the plant to produce grain (Sass et al., 1991b). Sass et al. (1992)
found that the loss of rice grain yield due to some stresses, such as late flooding, straw incorporation (possible damage of root by excessive organic acids), was accompanied with an increase in methane emissions and a decrease in rice yield.

To understand the plant physiological process of the nonstructural carbohydrate translocation and its influence on methane emission in rice fields, different stresses on rice plants were introduced into our experiments, i.e. root cutting and panicle cutting. Evidence is presented to suggest that there are some responses of rice plants to these artificial stresses in nonstructural carbohydrates translocation and, an accompanied increase in methane emission was observed. A hypothetical correlation between the possible loss of nonstructural carbohydrate (NC) due to the experimental manipulation of the rice plants and the increases of microbially driven methane emissions is proposed.
II. MATERIALS AND METHODS

2.1. Field Description

All the field work in this thesis was performed in rice fields at the Texas A&M University Agricultural Research and Extension Center near Beaumont, Texas, located at longitude 94°30'W, latitude 29°57'N. The local annual rainfall averages 1340 mm, approximately 50% (122 mm month⁻¹) occurring during the rice-growing season in April through September.

The soil type of the experimental fields was a Verland silty clay loam, classified as a fine montmorillonitic, thermic Vertic Ochraqualf. The soil is currently designated as Bernard-Morey and has a clay:sand:silt ratio of 24:30:46. The fields used were cropped with rice in the years before 1991 and fallow in 1991.

The rice cultivar used was 'Jasmine 85'. It is similar to the high tillering and high yielding rice grown throughout the developing world. 'Jasmine 85' was drill-planted at 112 kg ha⁻¹ in rows spaced 20 cm apart on April 23, 1992. The seedling density ranged from 250 to 300 m⁻². This cultivar was developed at the International Rice Research Institute, Los Baños, Philippines, and requires approximately 140 days to mature.
The fields were under normal management practices as that used in the commercial rice fields of the southern United States. They were permanently flooded on June 3, drained on September 3 and harvested on September 7.

2.2. Experiments

2.2.1. Methane Flux Measurement

Before flooding the fields, boardwalks were built across the field and 2 aluminum frames (0.397 m² in cross-section area) were installed on each side and near the end of the boardwalk in each field for the measurement of seasonal methane flux and emission. In the experimental field, 12 plastic collars, 24 cm in diameter, were set in the planting areas along the two sides of the boardwalk, with rice plants of a single 24 cm long row in each collar. About 8 cm of the collar wall was above the soil level in the field. When the field was flooded the collars were under water. Before the clipping treatments were initiated, flux measurements were conducted on the aluminum frame. Methane flux determinations were made by taking samples of the headspace gas in an open-bottom chamber of known cross-sectional area (0.397 m²) and volume (0.199 to 0.476 m³, depending on height of the flux box). When taking flux samples, the aluminum chamber was placed over the vegetation with the bottom edge of the chamber fitted into a groove in the aluminum frame and this chamber-frame system was air-tight sealed by filling the groove with water if the frame was not submerged. The chamber was thermally protected with an 1 cm thick
foam insulation on its top to minimize temperature changes during measurements. The chambers were fitted with a circulating fan to ensure complete gas mixing. Headspace air samples were taken from inside the box every 5 minutes for 25 minutes. Methane mixing ratios were determined with a gas chromatograph fitted with a flame ionization detector. Methane emission was determined from the slope of the mixing ratio change in the above set of five samples taken over a 30 minutes sampling period.

After the cuttings were made, flux measurements of the treatments were made by taking air samples mounted on the collars every 4 days with 0.0328 m³ plastic cylinders. The bottomless cylinder was placed on the collar with a groove and was air-tight sealed by filling the groove with water. The cylinders were shaded with thick foam insulation material. Five 50 cc air samples from the headspace were taken every 5 minutes. No fan was installed in the cylinder. To ensure complete gas mixing inside the cylinder, sampling syringe were pumped approximately 30 times before air samples were withdrawn.

Air temperature inside the chamber or cylinder, water temperature and temperature of 5 cm below soil surface were recorded at the time when flux samples were taken.

2.2.2. Soil Incubation Measurements
Methane production values were estimated by incubation of soil core segments of known cross-sectional area and depth collected within the rows of rice plants (Sass et al., 1990). Every week, triplicate soil cores of 10-cm-long and 2.8 cm in diameter were taken from each plot and each core was divided into four 2.5-cm-long segments, which represented the soil from different depth intervals of the field: 0 to 2.5 cm, 2.5 cm to 5 cm, 5 cm to 7.5 cm and 7.5 cm to 10 cm. They were then transferred to 60 cc syringes and the syringes were filled with the field water to 30 cc and sealed them with 3-way gas-tight stopcocks. Soil samples were then taken to the laboratory, purged and shaken 3 times with nitrogen to restore anaerobic condition, filled with nitrogen, and incubated at 28°C. Every 24 hours, these samples were taken out of the incubator and shaken vigorously for 5 minutes to strip the methane produced during the incubation period from the soil solution to the headspace and the headspace gas was examined for CH₄. After each run, the methane left in the soil samples was stripped with nitrogen and the anaerobic condition was restored before the soil samples were incubated.

2.2.3. Biomass and Total Nonstructural Carbohydrate (TNC) Samples

Changes in chemical composition of plant material are often caused by loss of moisture during sampling and preparation or loss due to respiration, decomposition by heat, or by enzymatic activity. However, the study of Fick et al. (1986) showed that TNC samples can be transported from the field to the drying oven without taking any precautions to prevent enzymatic degradation. Therefore, there was no special method for
preserving the plant samples in our experiments. Rice plants of a 50-cm-long row (20 cm between rows), equivalent to plants in about 0.1 m² vegetation area, were chosen randomly and dug out as one sample from the field every two weeks before heading. The plant roots were washed free of soil with water after removal from the field, transported to the laboratory in an ice chest with dry ice, then stored in a -10°C freezer in the laboratory.

Six different clipping treatments are described in Table 1, including

<table>
<thead>
<tr>
<th></th>
<th>No panicle cut (P⁻)</th>
<th>Half panicle cut (P¹/²⁺) (August 3, 1992)</th>
<th>Panicle cut (P⁺) (August 3, 1992)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No root cut (R⁻)</td>
<td>R⁻P⁻</td>
<td>R⁻P¹/²⁺</td>
<td>R⁻P⁺</td>
</tr>
<tr>
<td>Root cut (R⁺) (July 30, 1992)</td>
<td>R⁺P⁺</td>
<td>R⁺P¹/²⁺</td>
<td>R⁺P⁺</td>
</tr>
</tbody>
</table>

one control treatment, R⁻P⁻. Rice plants of a 24 cm long row were chosen for each sample. Root cut was committed on July 30 at the start of heading. The cuts were 3.75 cm away from the base of the plants and straightforward down to about 15 cm deep in the soil on each side of the row. At both ends of the chosen length in the row, cuts were made right at the base of the plants. Panicle cut was committed after heading on August 3. For half panicle cut, the panicles were cut right in the middle of panicle with scissors. For the whole panicle cut, instead of cutting the panicles above flag leaves, all the spikelets were stripped and left with very little
supporting structure. In order to identify different treatments and separate samples easily, color flags were placed beside each sample and encircled loosely together with the sample with cord. These clipping treatments were committed at the same time and in the same way to the plants in the 12 collars mentioned above.

Six experimental treatments were conducted in this experiment and two replicates were made for each treatment. A total of 72 plant samples were treated with root cut on July 30, 1992 or panicle removal on August 3, 1992, as described in Table 1. These samples were collected on 6 different sampling dates. On each of the sampling dates, two replicates of each treatments were collected.

2.3. Biomass Determination and Nonstructural Carbohydrate Detection

2.3.1. Live Biomass Determination:

Each plant sample was removed from the freezer and cut into 4 or 5 parts, i.e. root, leaf, stem, base and panicle. Here, culm and leaf sheaths were defined as stem. Base is the bottom 3 cm of the stem. Panicle is the upper part of plant from the first node above the flag leaf. During cutting, any dead part of the plants was removed. The leaf, stem, base and panicle were cleaned with water and the roots were washed free of soil. These samples were dried in a forced air oven at 75°C for approximately 40 hours and cooled to room temperature (20±2°C) for about 5 hours. During this cooling period the plant moisture content equilibrated with the
moisture in the air. The net weight of each part was weighed at this time. The sum of the dry weight of each part is the dry live biomass of the plant sample. The different parts were separately ground in a mini-mill with #10 mesh and followed by passing a #40 mesh sieve. One gram of each powder was placed in an aluminum tray and the tray was placed in a closed forced air oven at 80°C for 48 hours. These powder samples were transferred to a desiccator and weighed again with the samples staying in the open air as short a time as possible. The moisture content of the powders were thus determined.

2.3.2. Nonstructural Carbohydrate Determination

The five samples were treated with the hydrolytic enzyme "Clarase 40,000" (Milds Laboratories) and the nonstructural carbohydrates, such as starch, oligosaccharides, monosaccharides, were hydrolyzed to reducing sugars. Nonstructural carbohydrates can be determined by detecting the amount of reducing sugars. The procedure was that modified by the Texas A&M University Extension Station at Beaumont, Texas from Smith Dale's "Removing and Analyzing Total Nonstructural Carbohydrate from Plant Tissue" (University of Wisconsin Research Report No. 41, 1969). The procedure is as follow:

I. Chemicals and Reagents:

Buffer solution: Mixture of 1 liter of 0.2 N sodium acetate and 1.5 liters of acetic acid (pH 4.46).
Enzyme Solution: 2.5 g L\(^{-1}\) "Clarase 40,000".
Potassium Iodide-Potassium Oxalate: 25 g L\(^{-1}\).
10% Copper Sulfate Solution.
0.017N Potassium Iodate Solution (3.567 g in 1 liter water).
"Reagent 50": [50 g Na\(_2\)CO\(_3\)+50 g KNaC\(_4\)H\(_4\)O\(_6\).4H\(_2\)O+150 ml 10% CuSO\(_4\)+ 40 g NaHCO\(_3\)+2 g KI+400 ml 0.017 N KI\(_2\)O]/2 liter.
1 N H\(_2\)SO\(_4\).
Starch Solution: 1. Add 1 g soluble starch (rice starch) to 10 ml of cold distilled water;  2. Heat 100 ml distilled water to boiling and slowly add 1 g boric acid crystals;  3. Add starch solution, allow to boil for 1 minute and cool slowly to room temperature.
0.1 N storage Na\(_2\)SO\(_2\)O\(_3\), for titration, dilute to 0.02 N.
1 mg/ml Sugar standard: 1. Carefully dry ASC-grade glucose overnight in a forced air oven;  2. Weigh 1 g of glucose into a 1 L volumetric flask and bring to volume with a saturated solution of benzoic acid.

II. TNC Removal from Rice Plants

(a) Weigh 200 mg each of above plant powder into 50 ml Folin-Wu tube. Add 10 ml of distilled water in two aliquots to wash down the sides of the tubes. One tube containing only distilled water is included as an enzyme blank.

(b) Cover samples with foil caps and heat samples in water bath at 98-100°C for 15 minutes.
(c) Cool samples to below 30°C by placing in a sink of cold water for fifteen minutes (add ice when necessary).

(d) Add 10 ml buffer solution and 10 ml enzyme solution to each tube.

(e) Replace foil caps and incubate samples at 38°C for about 44 hours (Not to exceed 50 hours).

(f) After removing samples from incubator, filter through #1 Whatman paper into a 100 ml volumetric flask. Wash the filter paper and test tubes three times with distilled water.

(g) Bring to volume with distilled water and mix well. Store samples in refrigerator until titration.

III. Determining Reducing Sugars:*

(a) Remove samples from refrigerator and allow to reach room temperature. Pipette 5 ml of sample into Folin-Wu tubes. Also prepare the following controls:

Three reagent blanks of 5 ml distilled water.
Three enzyme blanks of 5 ml incubated enzyme solution.
Three glucose blanks using 2 ml glucose solution and 3 ml distilled water.

(b) Add 10 ml of "Reagent 50" to each of the above samples and heat for fifteen minutes in a water bath 100°C.

* Prior to the initiation of this nonstructural carbohydrate determination study, reagent concentration was tested. "Reagent 50" blanks should be approximately 9.9 ml 0.02 N Sodium Thiosulfate, Enzyme blanks 8.9 ml, and Glucose standard 5.1 ml.
(c) Cool in a container of cool water for 15 minutes or in ice water for 5 minutes.

(d) Add 2 ml of potassium iodide-potassium oxalate solution to each tube followed by 10 ml 1N H₂SO₄.

(e) Add 4 to 5 drops of starch solution as the indicator and titrate solution to a pale blue end-point with 0.02 N sodium thiosulfate.

Then, with the titration values,

\[
\text{% NC in a powder Sample} = (\text{Enzyme-Sample}) + ("\text{Reagent 50"}\text{-Glucose}) \times (2 \text{ mg glucose ml}^{-1}) \times (\text{dilution factor}) / (\text{sample weight}) \times 100.
\]

To convert to dry weight basis,

\[
\text{% NC of a sample on dry weight basis} = \text{% NC} + (1 - \text{Moisture % of the corresponding powder sample}).
\]

This method is good for any sample with quantity less than 4.4 mg of glucose or fructose per 10 ml of solution (Private Communication, Mike Jund). However, the enzyme may not hydrolyze fructosans. Sample that contains fructose or high levels of sucrose should be hydrolyzed with acid. Tests indicated that it is good for rice plant tissue carbohydrate analysis at any growth stage.

2.4. Instruments:

Gas Chromatography: Shimadzu GC-8AIF flame ionization gas chromatography.

Digital Titrator: 50 ml Brinkmanin Digital Buret.

Digital Thermometer with a probe connected with a cable.
III. RESULTS

3.1. Percentage Nonstructural Carbohydrate in Rice Plant Tissues

3.1.1. Root System

Figure 1(a) shows the percentage content of nonstructural carbohydrates (NC) in roots based on the dry root biomass. The NC in roots was high in the early vegetative phase and then began to decrease at panicle differentiation. It reached the minimum level in the ripening phase (about day 70). At the end of our observations, the NC in the roots increased in all experimental treatments. Although the amount of NC in the root system increased, the relative NC content decreased with the development of rice plants. This is probably because the increase in structural materials is more rapid than that of nonstructural materials. No statistical significant difference (all $P>0.3$) is observed between the NC content in the roots of the different experimental treatments early after cutting treatments, except the difference between R-P1/2+ (half panicle cut only) and R-P+ (whole panicle cut only) ($t$ test $P=0.03<0.05$) during the late ripening phase. This is probably because rice root is not a somatic storage tissue and has very low capacity to accumulate carbohydrates.

Figure 1(b) shows the differences of NC percentage content in roots among the experimental treatments of "root cut" and "no root cut" after cutting. Regardless of last data points, which are from the plant samples in the collars of different experimental treatments and are somehow different
FIGURE 1

NONSTRUCTURAL CARBOHYDRATE IN ROOT

(a)

Percentage Nonstructural Carbohydrate

(b)

Percentage Nonstructural Carbohydrates

DAYS FROM FLOODING
from the samples collected in other area of the field, when the panicle was intact or half cut, percentage NC content in the root tissues of the samples with root cut (R+) and no root (R-) cut did not show any significant difference (t test P>0.70) [Figure 1(b)]. However, when the whole panicle was cut, the NC content in treatments with no root cut is higher than those in treatments with root cut (t test P=0.10>0.05) [Figure 1(b)].

3.1.2. Stem and Base

The stem in our experiment includes both culm and leaf sheaths. Base is the bottom 3 cm of the stem. It is designated as a separate part to provide some information of the TNC gradient in the stem. In the early growth stage, before heading, the NC content in the stem and plant base of the normal plant increases with the development of the plant [Figure 2(a) and Figure 3(a)]. An NC gradient from the lower part to the upper part of the stems is gradually formed because the NC content in the plant base become higher than that in the stem. [Figure 2(a) and Figure 3(a)]. The NC content in the stem and base began to decrease rapidly after flowering initiated, i.e. the day when the panicle cut was made [Figure 2(a), 3(a)]. In the following two weeks it reached the minimum level and then became constant at the low level. During this period, the translocation of NC from stem, plant base and leaf to the panicles was finished. When the sink was limited by cutting half panicles (R-P1/2+ and R+P1/2+) [Figure 2(b), (c); 3(b), (c)], the NC content in stem and base decreased about one half of what it did in plants with no panicle cut. When the entire panicle was removed (R-P+ and R+P+), the NC content in the stem and base showed a
FIGURE 2
NONSTRUCTURAL CARBOHYDRATE IN STEM

(a) R-P-  
R-P1/2+  
R-P+  

(b) R-P-  
R-P1/2+  
R-P+  

(c) R+P-  
R+P1/2+  
R+P+  

DAYS FROM FLOODING
FIGURE 3
NONSTRUCTURAL CARBOHYDRATE IN PLANT BASE

(a) R+P+  R-P+  R-1/2P+
K-P-  R-P+  R+1/2P+

(b) R-P+  R-P-  R-P1/2+

(c) R+P+  R+P-  R+P1/2+

DAYS FROM FLOODING
general increasing trend during the whole season [Figure 2(b), (c); 3(b), (c)]. This can be interpreted as sink removal, i.e. as the panicle sink was removed the stem and base replaced the panicle as a sink which accumulated the NC from photosynthesis. The maximum NC content in the plant base with no panicle, either with roots cut or intact roots, is approximately the same level as that of the normal panicle [Figure 2(b), (c); 3(b), (c)]. Root cutting did not have a significant influence on the trends in NC content in either stem or plant base under conditions of no panicle cut, half panicle cut or whole panicle cut.

In cases where panicles were not cut or partially cut, cutting roots did not have a significant influence on the percentage NC levels in either stem or base [Figure 2(b), (c); Figure 3(b), (c)]. When the panicles were left intact, root cutting appeared to delay the decrease in percentage NC content in stem and base [Figure 2(a); Figure 3(a)]. Under condition of either root cut or no root cut, the NC levels in stem and base of the plants with partial or whole panicle cut are about 25% higher than those in plants with no panicle cut [Figure 2(b), (c); Figure 3(b)], although the difference between half panicle cut and whole panicle cut is not significant.

3.1.3. Leaf

Leaves are the primary source of all carbohydrates in rice plants. From Figure 4(a), one can see that in the early stage of development, the
FIGURE 4
NONSTRUCTURAL CARBOHYDRATE IN LEAF

(a) R+P+  
   R-P-  
   R-P1/2+  
   R-P+  
   R+P-  
   R+P1/2+  

Percentage Nonstructural Carbohydrate

(b) R-P-  
   R-P1/2+  
   R-P+  

(c) R+P-  
   R+P1/2+  
   R+P+  

DAYS FROM FLOODING
leaves grew rapidly while the percentage NC content reached a constant level during this period of time. Just as the root, rice plant leaf is not a somatic tissue for photosynthate storage. Normally the nonstructural carbohydrate content of leaves is relatively constant after they are mature because they translocate the photosynthates to stem immediately after they are made (Venkateswarlu et al., 1987). However, in the late phase of panicle ripening, the leaves have almost finished their function and begin to die or become less active in photosynthesis.

In the treatments with a whole panicle removal, half panicle cut or no panicle cut [Figure 5(a), (b), (c)], the percentage NC in leaves shows an irregular saw-tooth shape variation. However, in the treatments with intact panicles and panicle removal, the NC content in leaves of the root-cut plants was approximately 2-3% higher than that in plant with intact root [Figure 5(a), (c)]. There is no significant difference between the NC levels in the treatment with root cut and half panicle cut and the treatment with intact root and half panicle cut. Comparing the percentage NC level in the plants with the same root treatment but with different panicle treatments [Figure 4(b), (c)], it appears that the NC in the leaves is higher with correspondingly more severe panicle removal.

3.1.3. Panicle

In Figure 6, the control plant data show that the nonstructural carbohydrate content in the panicle increase rapidly after flowering and remained high (35%) during panicle maturation. In treatments with no panicle cut or half panicle cut, root cutting showed no significant effect on
FIGURE 5
NONSTRUCTURAL CARBOHYDRATE LEAF

(a) 
- O - R-P-
- O - R+P-

(b) 
- ■ - R-P1/2+
- □ - R+P1/2+

(c) 
- ■ - R-P+
- □ - R+P+

DAYS FROM FLOODING
FIGURE 6

PERCENTAGE NONSTRUCTURAL CARBOHYDRATE IN PANICLE

DAYS FROM FLOODING
NC content in the panicles (Figure 6). Although the NC levels in the two treatments of half panicle cut are lower than those in the treatments with no panicle cut in the early stage of panicle maturing, the NC level in the half panicles reached the same high level as that of the intact panicles at the end. Compared with the plants of no panicle cut, the percentage NC content in the panicles of half panicle cut showed a lower increase rate (Figure 6) when roots were either cut or not cut.

3.2. Total Nonstructural Carbohydrate Partition

3.2.1. Control (R-P-) and Root Cutting (R+P-)

Figure 7(a) shows that in the early stage of development of normal rice plants, most of the TNC* is contained in the stem and base. For the rice cultivar used in this experiment, NC storage in the stem continued to increase after panicle differentiation [Figure 7(a)]. When the NC is accumulated in the stem and base, the proportion of TNC partitioned in both leaf and root is almost constant [Figure 7(a)]. At heading, the proportion of TNC in stem and plant base began to decrease rapidly and the fraction of TNC in leaf and root did not change significantly [Figure 7(a)], however, the fraction of TNC in panicle increased rapidly as the grains began to fill. During this two weeks (day 60 to day 75), about 60% of the pre-heading NC storage in stem and base were translocated to panicle [Figure 7(a)]. The proportion of TNC in the panicle increased faster than the NC translocation from stem and base. During this period,

* TNC is the total nonstructural carbohydrates in the whole plants, i.e. the sum of the nonstructural carbohydrates (NC) in each part of the plants.
FIGURE 7

TOTAL NONSTRUCTURAL CARBOHYDRATE PARTITION

(a)  ROOT
     PLANT BASE
     STEM
     LEAF
     PANICLE

(b)  ROOT
     BASE
     STEM
     LEAF
     PANICLE

DAYS FROM FLOODING
NC/TNC ratio** of stem and base decreased 50% and 15%, respectively, when that of panicle increased 75%. The leaf photosynthesis may have compensated for this 10% difference. At the end of our observations, around 85% TNC in normal rice plants was partitioned to the panicles [Figure 7(a)]. Since the TNC, the denominator of NC/TNC ratio, increased greatly during the ripening phase because of the photosynthesis, the leaf photosynthesis should be responsible for more NC increase in the panicle than that from this simple calculation, 10% of TNC. This suggests that this rice cultivar is very efficient in nonstructural carbohydrates translocation and potentially high yielding. The TNC re-translocation process observed in the root cutting treatments but with no panicle removal [Figure 7(b)] is almost the same as in control plants. Therefore, a certain degree root cutting might not affect the translocation of TNC.

3.2.2. Normal Root with Half Panicle Removal (R-P1/2+) and Root Cutting with Half Panicle Removal (R+P1/2+)

When half of the panicle was removed, changes were observed in TNC translocation. Figure 8(a) and (b) suggest that half panicle removal altered the TNC mobilization from stem and base to the remaining portion of the panicle. The TNC partitioned in the stem and base still increased while the fraction of TNC in the panicle increased much more slowly than in the intact panicles, which suggests that more NC from leaf photosynthesis moved to the stem and base than left the stem and base to the

**NC/TNC ratio of a tissue is the fraction of TNC partitioned to the tissue.
FIGURE 8

TOTAL NONSTRUCTURAL CARBOHYDRATE PARTITION

(a) ROOT
■ PLANT BASE
● STEM
● LEAF
□ PANICLE

Fraction of Total Nonstructural Carbohydrate (%)

0 10 20 30 40 50 60 70 80 90 100

0 1 2 3 4 5 6 7 8 9 10

DAYS FROM FLOODING

(b) ROOT
■ BASE
● STEM
● LEAF
□ PANICLE

R-P1/2+ R+1/2P+
panicle. Such would lead to the accumulation of nonstructural carbohydrates in stem and base. During the panicle ripening period, leaf photosynthesis was totally responsible for the increase of TNC in stem and panicle because that in root, leaf and base did not change appreciably [Figure 8(a), (b)]. This situation lasted for about two weeks followed by the translocation of the TNC to the panicle and the fraction of TNC in the stem decreased rapidly. During the three weeks of panicle ripening, about 20 to 30% of the TNC in the plants moved from the stem to the panicle in the treatment with normal roots and one-half panicle removal (R-P1/2+) and the treatment with both root and panicle experimentally manipulated (R+P1/2+).

3.2.3. Normal Roots with Panicle Removal (R-P+) and Root Cutting with Panicle Removal (R+P+)

When the whole panicle was removed, the nonstructural carbohydrate in the stem continued to increase throughout the season [Figure 9(a), (b)]. Accompanying this, the proportion of TNC partitioned to base, leaf and root did not change. The NC/TNC ratio for the base is largely influenced by the dry weight of base which is very low because it is only 3 cm in length, less than 10% of the total plant stem. The NC/TNC ratio for the plant base changes little when the percentage NC content in base increased. Here, the maximum of the NC/TNC ratio for stem is about the same as that of normal panicles [Figure 7(a), (b)], which suggests that the stem became the major carbohydrate sink after cutting the panicles.
FIGURE 9
TOTAL NONSTRUCTURAL CARBOHYDRATE PARTITION

(a) ROOT
   PLANT BASE
   STEM
   LEAF
   PANICLE

(b) ROOT
   PLANT BASE
   STEM
   LEAF

DAYS FROM FLOODING
The root cutting did not show any significant influence on the translocation of TNC and the pattern of TNC partitioning of rice plant in this study.

During the post-cutting period of the season, the NC/TNC ratio of leaf decreased the most rapidly in the treatments with intact panicles [Figure 7(a), (b)]. The NC/TNC ratio in the treatments with one-half panicle removed remained a relatively high level (10%) until day 82 [Figure 8(a)] and day 76 [Figure 8(b)] and then began to decrease. In the treatments with entire panicle removal, no appreciable decrease in NC/TNC ratio of leaf was observed [Figure 9(a), (b)]. This suggests that the more panicle removal, the less rapidly the NC/TNC ratio of leaf decreases during the grain maturation.

3.3. Methane Production and Emission

3.3.1. Production from Soil Incubations

Figure 10(a) shows the seasonal variation of methane production from soil incubations. In the top soil depth interval, 0-2.5 cm, three peaks of methane production were observed in the late vegetative stage (day 35), early productive stage (day 55) and the late ripening stage (day 75), respectively. The methane production curve for soil depth interval 2.5-5.0 cm is similar in shape to that of 0-2.5 cm soil depth interval in the first 2/3 season. In the late season, instead of a peak of methane production in 0-2.5 cm soil depth interval, a plateau was observed in the soil depth interval 2.5-5 cm. In soil depth interval 5-7.5, two peak methane productions were observed at the late vegetative stage (day 41) and late stage of grain
maturation (day 75) and generally, the methane production in this soil layer is much less than those in soil depth interval 0-2.5 cm and 2.5-5 cm. There was no significant variation of methane production in the soil depth of 7.5-10 cm. During the first two weeks after flooding, there was no significant difference in the methane production of different soil depth intervals [Figure 10(a)]. This implies the homogeneity of the availability of methanogenesis substrates in the soil cross-section during this early period of time. With the growth of rice plants, the methane production in the upper 5 cm is much higher than the lower 5 cm of the soil cross section, which means the main carbon source for the emitted methane is in the top 5 cm of the soil cross section where most of the roots are located.

3.3.2. Seasonal Variation of Methane Emission

A seasonal variation of methane emission rate from the field was measured [Figure 10(b)] with the metal flux box and permanently installed frame. The seasonal variation of methane emission in our observations could be characterized as a big mid-seasonal plateau [Figure 10(b)]. The flux from the collars of the control treatment measured with the cylinder-collar gas sample collecting system is about half that of measurements using the metal box-frame gas collecting system in the normal planting area in the same field on the same day. The first maximum of methane emission in our field was observed in the period of the rapid increase of the total dry biomass [Figure 10(c)], which was the active vegetative stage. This pattern of seasonal variation of methane emission is quite different from those of previous years in the fields with the same soil (Bernard-Morey).
10(b) shows that the methane emission rate increased rapidly to a high level in a much shorter period of time after normal flooding than those observed in 1989, 1990 and in some fields in 1991. The pronounced maximum of emission rate (689 mg m⁻² day⁻¹) in these observations is much higher than those observed in the same soil in 1989 and 1990, which were around 500 mg m⁻² day⁻¹ and similar to that observed in 1991. Different from any of the previous years, a flux plateau was observed and the maximum emission rate occurred in the early season instead of at the end of panicle ripening in 1989 and 1990 and at the reproductive stage in 1991. The daily average methane emission in this season is 352 mg m⁻² d⁻¹, compared to those observed in 1989, 1990 and 1991, i.e. 59.9, 134.2 and 106.54 mg m⁻² d⁻¹, respectively.

3.3.3. Methane Flux from the Cylinder-Collar

Flux from the twelve collars of six different treatments were measured, starting thirteen days after root cutting and 10 days after panicle cutting, during the last three weeks of the experimental period. Variation of methane emission rates were observed in all the six treatments during the post-cutting season [Figure 11 (a)]. There were pronounced increases in methane emission rates from the collars with plant manipulation relative to that from the control collars. Statistical analysis shows significant difference between the flux from control collars (R·P⁻) and the others (t test P<0.01) (Table 2). The difference between the fluxes of each two
FIGURE 11
DAILY AVERAGE FLUX FROM COLLARS

FLUX FROM COLLARS

DAYS FROM NORMAL FLOODING
treatments with different root cutting but with the same panicle manipulation, i.e. one-half panicle removal or entire panicle removal, is not significant (P>0.05). The fluxes of the treatments with the entire panicle removed are significantly different from those of the treatments with intact panicle or only one-half panicle removed (P<0.05), except those between the treatments "cut root with entire panicle removed" (R+P+) and "normal root with one-half panicle removed" (R-P^{1/2+}) (P=0.099). The daily average methane emission rates is shown in Figure 11(b). Relative to the flux from the control collars, there was an 1 to 3 times increase in the

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R\cdot P^{1/2+}</th>
<th>R\cdot P^+</th>
<th>R+P^-</th>
<th>R\cdot P^{1/2+}</th>
<th>R+P^+</th>
</tr>
</thead>
<tbody>
<tr>
<td>R\cdot P^-</td>
<td>0.001</td>
<td>0.001</td>
<td>0.000</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>R\cdot P^{1/2+}</td>
<td>0.047</td>
<td>0.200</td>
<td>0.171</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>R\cdot P^+</td>
<td>0.017</td>
<td>0.021</td>
<td>0.671</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>R+P^-</td>
<td>0.711</td>
<td>0.022</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R+P^{1/2+}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. P Values for t Test of the Difference Between the Flux Data from the Collars

daily emission rates in the collars with cutting treatments. The lowest emission rates occurred on August 18 (day 76) when the soil temperature at 5-cm depth was the lowest (22.1°C). This is consistent with the point that the methane emission rates are positively correlated with and sensitive to soil temperature, documented by some previous investigators (e.g. Schütz et al. 1989; Sass et al., 1991a, b). Fluxes from the collars varied with the soil temperature during the post-cutting observation [Figure 11(a) and Figure 12(c)]. Methane flux from all collars with cutting treatments is much higher than that from the control collars. Further more, the increase
of methane emission rate of whole panicle cut treatment is higher than that of half panicle cut treatments and much higher than that of no panicle cut treatments. The methane increases of the treatments with plant manipulation are significant and most of the daily emission rates observed in these treatments are higher than the normal seasonal maximum emission rate. The daily average emission rates from the collars [Figure 11(a) and (b)], the seasonal daily average emission rate [Figure 10(b)] and the daily average emission rate during the plateau period [Figure 10(b)] summarized are in Table 3. In table 3, some of the daily average methane emission rates of the collars with plant cutting are about twice, some are three times and some are even four times as much as that of control. The daily

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CH₄ Emission (mg m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R⁻P⁻</td>
<td>155.38</td>
</tr>
<tr>
<td>R⁺P⁻</td>
<td>398.17</td>
</tr>
<tr>
<td>R⁻P₁/₂⁺</td>
<td>495.56</td>
</tr>
<tr>
<td>R⁺P₁/₂⁺</td>
<td>422.47</td>
</tr>
<tr>
<td>R⁻P⁺</td>
<td>638.36</td>
</tr>
<tr>
<td>R⁺P⁺</td>
<td>603.82</td>
</tr>
<tr>
<td>Seasonal Daily Average</td>
<td>352.00</td>
</tr>
<tr>
<td>Daily Average of the Plateau</td>
<td>567.00</td>
</tr>
</tbody>
</table>

average CH₄ emission rate of the treatments with whole panicle cuts are even higher than the daily average of the control during the plateau period in the normal seasonal variation.
IV. DISCUSSION

4.1. Nonstructural Carbohydrate Translocation

Through the analysis of the TNC seasonal variation in each rice plant tissue, the carbohydrate translocation process in a normal rice plant during the growing season is presented [Figure 7(a), (b)]. Like other crops, the physiological basis of dry matter production for rice is dependent on the source-sink concept, where the source is the potential capacity for photosynthesis and the sink is the potential capacity to utilize or store the photosynthetic products. The source-sink relationship for rice changes with the development of rice (Venkateswarlu et al., 1987). In the early development stage, rice leaves are a sink as well as the source of carbohydrates, however, in the mature plant leaves are the source of carbohydrates. Developing buds and meristematic regions in root place demands on the available assimilates and compete successfully as sinks with developing leaves. During the vegetative period, the stem, consisting mainly the leaf sheaths, is the major sink of photosynthates. In the following reproductive stage, the stem becomes source as it provides the panicle with most of the nonstructural carbohydrates needed for heading and grain filling. In some rice cultivars, the stem (culm and leaf sheaths) store up to 90% (Yoshida, 1972) of the nonstructural carbohydrates for the ripening of grains during the preheading period. It was observed in our experiments that the accumulated nonstructural carbohydrates in the stems and bases began to move to the panicle several days after heading began.
The NC reserves in stem and base tissues provided most of the carbohydrates needed to fill the grains. During this time, the storage NC in the stem and base, together with the NC from leaf photosynthesis is mobilized to the panicles and the grains filled. The fraction of TNC in the panicles increased rapidly during this filling period and then continued to increase through importing photosynthate from the leaves.

In the half panicle cut treatments, TNC re-partitioning was also observed [Figure 8(a), (b)], but much less NC in the stem and base tissues was translocated to the panicles than that in plants with intact panicles and the carbohydrate reserves in stem began to decrease nearly two weeks later than that in control. The NC in the base was constant and even had increased somewhat during the panicle ripening stage. This indicates that the stem and base accumulated some nonstructural carbohydrates after the grains were filled.

In treatments with panicle removal [Figure 9(a), (b)], no nonstructural carbohydrate mobilization was observed. Taking the place of panicle, the stem and base continued to accumulate carbohydrates from the leaf photosynthesis. The nonstructural carbohydrate accumulating during the two weeks after heading was higher than that in any other time of the season. This suggests that natural chemical growth regulation in normal plants, which stimulates the leaf photosynthesis, might still exist in the early productive stage in the plants with the whole panicle removed.
Generally, cutting half or the whole panicle resulted in incomplete or no carbohydrate translocation and higher nonstructural carbohydrate levels were observed in stems and plant bases. These results suggest that on one hand, stem and plant base have a larger potential of accumulating carbohydrates than present in normal plants and, on the other hand, chemical signals functioning as growth regulators in normal rice plants during productive stage might have been released from the panicles before the panicles were cut in our experiments.

In four of the six treatments, a pronounced drop of the fraction of TNC in panicle and the corresponding increase in the stem and base were observed in the final three days of our observation (Figure 7 and 8). A slight drop of percent TNC in panicle and an obvious increase in the culm were also observed in the last week of growing season in some other investigations (e.g. Andrews, 1990). This is likely because of the damage of grains by the animals like birds and mice and correspondingly a larger proportion TNC is located in base and stem. When the grains are filled, the accumulation of carbohydrates in stem and base is also possible because some of the green rice leaves might still be photosynthesizing actively. During the late period of the season, the carbohydrates in grains may also be transformed to other forms that can not be detected with the methods employed in this research. However, the percent NC in the dry materials of both stem and base increased shortly during the last three days of the season, which is difficult to explain. The last data points were from the plant samples in the flux measurement collars. Visually, these plants were somewhat different from the plants taken from the central part of the field,
where the plants were less disturbed. The 8 cm underground collar might also affect the exchange of nutritional material and water between the rhizosphere soil and the soil outside of the collars. These might all affect the growth stage of the plants in these collars.

4.2. Percentage Nonstructural Carbohydrate in Leaf

The percent NC in leaf tissue presents a saw-tooth shape in each treatment. It was considered that sugar movement into plant cells through the phloem elements in the vascular strands was strictly a downhill diffusion (Mason et al., 1957; Swason, 1959), which is mostly a physical chemical process. Figure 12(a) demonstrates that when the soil/water temperature became higher the percent NC content in leaf tissue was lower and vise versa. This reverse correlation of the soil/water temperature and the NC content in leaf tissue suggests that the translocation of the assimilates from leaf photosynthesis might be sensitive to the environmental temperature.

In plants with intact panicle, leaf tissue also showed a decrease in the fraction of TNC from approximately 0.10 to 0.05 during the ripening phase, which was also observed in Andrews' (1990) study. Similar to Andrews' observation, it was also observed in our experiments that the percent TNC in normal rice leaves began to decrease about 10 days after the percent TNC in stem began to decrease [Figure 7(a), (b)]. In treatments with half or whole panicle removal, the percent TNC in the leaf began to decrease at late stages of grain maturation. However, in Figure
4(a), (b) and Figure 5(a), (b) and (c), there is a suggestion, albeit slight, that the percent NC contents of the leaf tissues in the samples with root or panicle cut are higher than that of normal plants and the more cut the higher is the percent NC content in the leaf tissue. Rice leaf is an assimilating tissue and the photosynthates produced in the rice leaf are usually transferred to the phloem in the vascular strands of leaf sheaths without a delay (Venkateswarlu et al., 1987). Under the same conditions, more nonstructural carbohydrate content in the rice leaf may mean a previously higher photosynthetic rate in the leaf. This suggests that the root cut and panicle cut did not depress photosynthesis in the rice plant leaves, but might have stimulated photosynthetic activity of the leaf tissue. Visual inspection suggested that leaves of the plants with experimental manipulation, particularly panicle removal, were greener than those of the normal plants. This might be because of more chloroplast produced in leaves. It is reported that the photosynthetic rate of wheat leaves responds to the demand for assimilates (King et al., 1967). During grain filling, when most of the assimilates from the wheat flag leaf are translocated to the ear, removal of the ear, i.e. cutting off the pathway for the assimilates to move to grains, leads to an accumulation of assimilates in the flag leaf and to a pronounced fall in its photosynthetic rate within hours. But, if the lower leaves are shaded so that the flag leaf has to support the rest of the plant, the photosynthetic rate rises to its original level (King et al., 1967). From our data, the stem and base had quite large potential capacities to accumulate carbohydrate from the leaves (Figure 8; Figure 9). The need for assimilates in the stem and base and the need for the formation of root exudates by roots (Sadhu et al., 1969) were perhaps a driving force for
intensified photosynthesis. However, other possibilities cannot be ruled out, such as feedback from plant chemical regulation., including hormonal regulation.

4.3. Percentage Nonstructural Carbohydrate in Root

The roots reached their maturity at about the time of panicle differentiation (P.D.) (Murata et al., 1975). In the subsequent reproductive phase, rice plants are physiologically most active and the rapid decrease of percent NC in the roots during this period of time is most likely because of the frequent material exchange between roots and the rhizosphere soil solution, which means more carbohydrate consumption for the higher energy load. This may result in more root exudates.

Generally, the fraction of TNC partitioned in roots in the treatments with no root cut showed no significant change in almost all treatments after panicle cutting while in treatments with root cutting, all treatments showed an increase, this observation is hard to explain. However, the fraction of TNC partitioned in the roots in treatments with normal roots but different panicle manipulations are larger that that in the treatment with normal root and intact panicle [Figure 7(a) and 8(a) and 9(a)]. The same situation was found in the treatments with cut root and different panicle treatments [Figure 7(b) and 8(b) and 9(b)]. These were not because of the mathematical re-partitioning, i.e. a smaller denominator (TNC) because of one-half or entire panicle removal, but the physiological re-translocation of carbohydrates. Therefore, partial or total panicle cut might have caused
more TNC to be partitioned to roots and the early side root cut somehow induced some buildup of TNC in the roots. Thus, both panicle cut (partial or total) and root cut might have given some direction, downward to roots, if not the driving force, for the movement of nonstructural carbohydrates. However, rice roots are not a storage tissue and no significant amount of nonstructural carbohydrate accumulation was observed in this tissue. This means that in addition to the normal formation of root exudates, some of the nonstructural carbohydrates might be lost through the roots due to the experimental plant manipulation.

Figure 12(b) suggests a possible positive correlation between the soil/water temperature and percent NC content in roots. This correlation is particularly apparent in plants with treatments R·P-, R·P1/2+ and R+P1/2+. Although this correlation is not pronounced, it implies the possibility that soil temperature affects the methane emission from rice field through influencing the formation rate of root exudates, as well as through other mechanisms.

4.4. Possible Loss of Nonstructural Carbohydrates Due to Experimental Plant Manipulation

To evaluate the TNC accumulation in plants of different treatments during the post-cutting period of the season, a criterion to measure the change of nonstructural carbohydrate is particularly necessary. Although an effort was made when plant samples were taken for nonstructural carbohydrate analysis, it is hard to collect the same amount of plants in the
samples because of many factors, such as the seed density, number of tillers, different growth stage of the tillers, the death of leaf sheaths and leaves in the late season and the removal of plants etc. Everything in rice plants was changing during the entire season. Therefore, the TNC in the collected samples may be affected by many factors, such as death, etc. However, in the late period of the season, especially after flowering, the structural part of the stem and base relatively may be the most stable part and was affected least by the above factors. Using the structural part of the stem and base (SBS) as the denominator, the trends of nonstructural carbohydrates can be seen in the change of TNC/SBS ratio. Figure 13 shows the general trends of increase of TNC/SBS ratio of no panicle cut treatments and the TNC/SBS ratios of half panicle cut treatments are basically increasing but at low rates. In the treatments with whole panicle cut the TNC/SBS ratios do not show an increasing trend, but rather a decreasing trend. Linear regression shows that each treatment has a different slope that represents the increasing of TNC/SBS ratio and indirectly represents the TNC increase in each plant treatment. The treatments with no panicle cut have the two largest positive slopes that are very similar while the half panicle cut treatments have much smaller and different positive slopes (Table 4). The whole panicle cut treatments even have negative slopes with very close absolute values. This again indicates that the TNC in rice plants is sensitive to panicle cut but not to root cuts. The changes of the TNC/SBS ratios of these treatments during the post-cutting season, again, strongly suggest that rice plants may lose some of their nonstructural carbohydrates due to the cutting treatments. The most
FIGURE 13
TOTAL NONSTRUCTURAL CARBOHYDRATE AGAINST STRUCTURAL PART OF THE STEM AND PLANT BASE

(a)  
- R-P-  \( y = -116.40 + 2.203x \)  \( R^2 = 0.889 \)
- R-P1/2+  \( y = 24.67 + 0.329x \)  \( R^2 = 0.272 \)
- R-P+  \( y = 58.13 - 0.306x \)  \( R^2 = 0.204 \)

(b)  
- R+P-  \( y = -91.15 + 1.925x \)  \( R^2 = 0.628 \)
- R+P1/2+  \( y = 2.968 + 0.649x \)  \( R^2 = 0.629 \)
- R+P+  \( y = 53.39 - 0.183x \)  \( R^2 = 0.116 \)

DAYS FROM FLOODING
probable place for the lost NC or its metabolic products is into the root-soil system.

The statistical description of the differences among the various TNC/SBS ratios are as Table 4.2. The treatments root cut with panicle removal (R+P+) and normal root with panicle removal (R·P+) have a statistically significant difference (P<0.05) from all the other 5 treatments. Eleven of the above 15 pairs of treatments have statistical differences, i.e. P<0.05. Basically, the experimental plant manipulation might have made some significant influence on the TNC accumulations of rice plants.

Table 4. The Confidence Level of the Difference among the TNC/SBS Ratios
(P value for t Test)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R·P^{1/2+}</th>
<th>R·P+</th>
<th>R+P-</th>
<th>R+P^{1/2+}</th>
<th>R+P+</th>
</tr>
</thead>
<tbody>
<tr>
<td>R·P-</td>
<td>0.420</td>
<td>0.045</td>
<td>0.044</td>
<td>0.032</td>
<td>0.048</td>
</tr>
<tr>
<td>R·P^{1/2+}</td>
<td></td>
<td>0.002</td>
<td>0.196</td>
<td>0.072</td>
<td>0.004</td>
</tr>
<tr>
<td>R·P+</td>
<td></td>
<td></td>
<td>0.028</td>
<td>0.003</td>
<td>0.033</td>
</tr>
<tr>
<td>R+P-</td>
<td></td>
<td></td>
<td></td>
<td>0.346</td>
<td>0.046</td>
</tr>
<tr>
<td>R+P^{1/2+}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
</tbody>
</table>

The TNC increase in each treatment (including control) is calculated by using the slopes in Figure 6(a) and (b) and the change in the structural materials in the plant base and stem. The estimated TNC losses relative to control in the post-cutting period of the season are shown in Table 5.
Table 5. Estimation of TNC Loss in the Treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Slope</th>
<th>TNC Increase [g m⁻² d⁻¹]</th>
<th>TNC Loss [g m⁻² d⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control [R-P⁺]</td>
<td>2.203</td>
<td>13.258</td>
<td>0.000</td>
</tr>
<tr>
<td>R+P⁻</td>
<td>1.925</td>
<td>10.689</td>
<td>2.569</td>
</tr>
<tr>
<td>R+P¹/₂⁺</td>
<td>0.628</td>
<td>3.907</td>
<td>9.351</td>
</tr>
<tr>
<td>R⁻P¹/₂⁺</td>
<td>0.329</td>
<td>1.518</td>
<td>11.740</td>
</tr>
<tr>
<td>R+P⁺</td>
<td>-0.183</td>
<td>-0.571</td>
<td>13.828</td>
</tr>
<tr>
<td>R⁻P⁺</td>
<td>-0.306</td>
<td>-2.044</td>
<td>15.303</td>
</tr>
</tbody>
</table>

4.5. Seasonality of Methane Flux and Production

A plateau of seasonal methane emission was observed in our field [Figure 10(b)]. Two methane emission peaks were observed in 1989 and 1990 (Sass et al., 1990, 1991a). One peak or multiple emission peaks were observed in 1991 (Sass et al., 1991b). The methane emission rate increased to a high level (689 mg m⁻² d⁻¹) much faster than in previous observations in fields with the same soil. Bare plot experiments (Sass et al., 1990) indicated that significant methane emission was only observed in the first three weeks after flooding. Methane production in the first 3 weeks after normal flood is believed to be caused by the mineralization of organic matter in the form of rice straw and stubbles present in the soil before flooding (Holzapfel-Pschorr et al., 1986; Schütz et al, 1989a; Sass et al., 1990). However, our experiment fields was fallow in 1991 and there was no appreciable accumulation of organic matter for the first peak of methane emission. The first maximum of methane production overlaps
quite well the active vegetative stage and the second overlaps partially the reproductive stage of rice plants [Figure 10(b), (c)]. The root system reached maturity at approximately the time panicle differentiation was finished. This suggests that the root system might have also played an important role as a main carbon source for methanogenesis during the early season. Some research on root exudates of plants like alfalfa (Hamlen et al., 1972) shows that the age and stage of development significantly affects the qualitative and quantitative nature of plant root exudates. Flowering plants produce significant amounts of materials released to the soil or cultural solution through roots. During grain filling, a large amount of nonstructural carbohydrates is needed by each part of the plant because of the high energy need for absorbing nutrients from soil and/or photosynthate transformation and transportation. At this time metabolites from nonstructural carbohydrates would increase and the formation of root exudates might also increase. However, a middle season minimum of methane production was found [Figure 10(a)], which is left unexplainable. Following the grain filling stage, the physiological activities of the ripening rice plants are the transformation of sugars to starch and the loss of moisture. During this period of time, there was no more carbohydrate translocated to panicle and more NC could be available for the formation of root exudates, which may result in more RMC*** and subsequently more methane production accounting for the third maximum of methane production [Figure 10(a)].

It has been observed that the seasonal pattern of CH₄ emission rates differed within an individual year and from year to year in Italian rice fields (Schütz et al., 1989). Seasonal methane flux measurements from European (Schütz et al., 1989) and United States rice fields show a general correlation with soil temperature as well as the seasonal trends in plant development (Sass et al., 1990). With the growth of rice plants, the photosynthates from the rice leaves, particularly carbohydrates, are translocated to every part of the plant. Some carbohydrates and metabolic substrates translocated to the root system leak out as root exudates. These normally contain carbohydrates, organic acids and amino acids (Vancura and Hovadik, 1965; Boureau, 1977). Root exudates are the primary source of carbon for the methanogenic bacteria, although there are also two other possible carbon sources for the methanogens, i.e. organic compounds in the soil and flood water biomass like algae. However, that the application of rice straw resulted in an increase in CH₄ emission mainly during the early season (Sass et al. 1989; 1990) suggests that the carbon source for most of the seasonal methane production is probably from the root exudates and substrates from root decay which are originally from the photosynthates of the leaves of living rice plants. Since methane emission is the overall result of processes of production, oxidation and transport, it could be affected by many factors. Therefore, the regime of seasonal temperature in soil, the nitrogen and sulphur conditions in soil and the organic carbon sources other than root exudates and root decay, such as the organic materials from algae, could all affect methane emission rates and all of these are usually subject to change from year to year and from field to field because of rice cultivation management, soil nutritional condition and weather variation.
4.6. Methane Fluxes of the Different Plant Treatments and a Hypothesis

As demonstrated in Figure 11(b), there are significant increases in the methane emission rates in the collars with cut plants. There is a significant difference between the flux of collars with whole panicle cut and those with no or half panicle cut. But root cutting did not show any significant effect on methane emission rates. Two weeks might be enough time for the regeneration of roots. In addition, the root ends were located in the upper layer of soil or floating in the water. These roots might have been decomposed by aerobic bacteria when the flux was measured.

Evidence is reported that a decrease in rice yield due to some stresses to rice plants resulted correspondingly in an increase of methane emission, and an increase in rice grain yield was accompanied with a decrease in methane emission (Sass et al., 1991a). Taking methane oxidation into consideration, a loss of 1 g m⁻² of simple carbohydrate from the rice plant to the rhizosphere corresponds to a predicted increase in methane emission of 0.111 g m⁻² (Sass et al., 1991a). Following this calculation, if the increases of the methane emissions observed in the collars were totally derived from the TNC that the cut plants somehow lost, the TNC accounting for the increased methane emissions would be as listed in Table 6. The correlation of the estimated TNC loss and the TNC accounting for
Table 6. Predicted TNC Accounting for the Increase of Methane Emission

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TNC Needed (g m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (R⁻P⁻)</td>
<td>0.000</td>
</tr>
<tr>
<td>R⁻P¹/₂+</td>
<td>3.530</td>
</tr>
<tr>
<td>R⁺P⁺</td>
<td>5.006</td>
</tr>
<tr>
<td>R⁺P⁻</td>
<td>2.523</td>
</tr>
<tr>
<td>R⁺P¹/₂+</td>
<td>2.774</td>
</tr>
<tr>
<td>R⁺P⁺</td>
<td>4.649</td>
</tr>
</tbody>
</table>

the increased methane emissions is shown in Figure 14(a). Figure 14(a) shows, with a high confidence level (t test P=0.007), that there is somehow a strong correlation between the estimated TNC losses and TNC needed for the increased methane emissions. The regression and statistical test results are summarized in Table 7. Even without taking consideration of the origin point, which implies that when there is no carbohydrate loss relative to control, there is no increase in methane emission, the regression analysis

Table 7. Regression Analysis Results in Figure 14

<table>
<thead>
<tr>
<th>Including the Original Point</th>
<th>Excluding the Original Point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression Equation</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Figure 14(a)</td>
<td>y=0.700+0.270x</td>
</tr>
<tr>
<td>Figure 14(b)</td>
<td>y=0.042+0.371x</td>
</tr>
<tr>
<td>Figure 14(c)</td>
<td>y=0.122+0.188x</td>
</tr>
</tbody>
</table>
FIGURE 14
"TNC LOSS" vs "TNC ACCOUNTING FOR THE INCREASE OF METHANE EMISSION"

(a)

(b)

(c)

ESTIMATED TNC LOSS (g/m^2/day)
presents a positive correlation with a confidence level of $P=0.044$. This suggests that a significant amount of nonstructural carbohydrates or its metabolites was most likely lost through the rice plants in the form of root exudates due to the experimental plant manipulation. These lost carbohydrates might then become the methane emitted from the field. This also suggests that there is a possible competition for the nonstructural carbohydrate between rice panicles and the bacteria during the normal growth of rice plants.

Actually, in Figure 14(a), the six treatments fall into three groups, A, B and C, according to the distance on the X-axis between each two data points, which means the difference of amount of TNC loss: A — whole panicle cut; B — half panicle cut; C — no panicle cut. This implies that the root cut has the least effect on TNC loss and the more panicle cut, the more TNC loss. Since root cut is only 5 cm away from the base of rice plants and was made when the root was fully developed, it is not expected to have much influence on plants. However, there is some difference between the treatment with root cut and that with no root cut, i.e. control (R-P-) compared with root cut with intact panicle (R+P-), normal root with one-half panicle removal (R-P1/2+) compared with root cut and one-half panicle removal (R+P1/2+), and normal root with whole panicle removal (R-P+) compared with root cut and whole panicle removal (R+P+) [Figure 14(a)]. The reproductive stage is physiologically the most active phase of rice plant growth and the root cut is no doubt a damage in some degree to rice plants and somehow affects the physiological activity of the rhizosphere. Panicle cut might have reduced the driving force of upward
carbohydrate movement and increased the downward movement of the accumulated carbohydrate in the stem and base, which then resulted in the formation of more root exudates.

The methane flux in the normal plant area of the field was also measured with the metal flux box on three separate days when the flux were measured with the collar-cylinder. The result in Table 6 shows that the flux from normal plants measured with the metal box is almost twice (1.956) as much as that from collars measured with cylinders. If the difference between the two flux measurements existed for every collar, the collar flux data could be corrected by the factor 1.956. However, the increases in methane emission rates over the control value are even larger and the correlation between the estimated TNC loss and the TNC calculated to account for the increased methane emissions is even stronger, because the linear regression line of the data points, excluding the origin point that represents the fact, almost passes through the origin [Figure 14(b)].

Field visual inspection of the plants in the collars indicates that the plants in the control collars were less robust than the plants in other collars and the normal plants in other area of the same field. Some investigators (e.g. Sass et al., 1990) have documented that plant density and above ground biomass have significant influence on methane emission rate and the methane emission rate also shows a positive relationship with root biomass. The pronounced difference between the fluxes with normal plants measured with the two different collecting containers is likely due to
Table 8. Flux from Control Plants Measured With Box-Frame and Collar-Cylinder

<table>
<thead>
<tr>
<th>Date</th>
<th>Day Since Flooding</th>
<th>Metal Box (MB) (mg m-2 d-1)</th>
<th>Cylinder-Collar (CC) (mg m-2 d-1)</th>
<th>MB/CC Ratio</th>
<th>Average Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/28/92</td>
<td>55</td>
<td>492</td>
<td>275.57</td>
<td>1.785</td>
<td>1.956</td>
</tr>
<tr>
<td>8/4/92</td>
<td>62</td>
<td>535</td>
<td>289.96</td>
<td>1.845</td>
<td>STDEV</td>
</tr>
<tr>
<td>8/18/92</td>
<td>74</td>
<td>287</td>
<td>128.25</td>
<td>2.238</td>
<td>0.246</td>
</tr>
</tbody>
</table>

differences in plant robustness. If we only correct the flux data from control collars, following the same calculations, the correlation of the estimated TNC loss and the TNC needed for the increased methane emission hardly changes from Figure 14(b) [Figure 14(c)]. Figure 14(b) and (c) indicate that the correction of these data do not negate the hypothetical correlation between the increased methane emissions and the estimated TNC loss from the changes of nonstructural carbohydrate translocation in the plants with root or panicle cut.

However, the increased methane emissions in the treatments are much less than that expected if the estimated TNC loss were completely converted to methane. The relative low soil temperature (about 4°C lower) in the late season might be a major factor limiting the effectiveness of using the carbon source by the bacteria. It is reported that larger proportion of methane produced in the paddy soil are oxidized in the late season than in the early season (Sass et al., 1992). Although an argument against this may be that the field soil temperature in the late season (22±2°C) is much lower than the laboratory production incubation temperature 28°C, which means that much less RMC is available in field soil at 22±2°C than in laboratory incubation soil at 28°C. It is still not
clear whether smaller or larger proportion of the methane produced in soil is oxidized in the ripening phase. More TNC would be needed for the increase of methane emission if a larger proportion, rather than seasonal average 60%, of produced methane were oxidized. In addition, instead of form of root exudates, some of the carbohydrates that were to fill the grains in normal plants might also transform to structural material due to cutting treatments. In fact, stems looked more robust and new tillers were observed in the collars with cutting treatments.

There seems to be a paradox between increasing rice grain yield and mitigating methane emission. Rice plants usually need high temperature and solar radiation during their reproductive and grain ripening stages. High solar radiation is required to meet the need for photosynthates by the grain filling process and high temperature is favorable for the translocation of photosynthates from leaf to grains. However, enough evidence shows that high temperatures tend to produce more root exudate (Bokhari et al., 1974). On the one hand, this may provide more carbohydrate for the soil bacteria, on the other hand, high temperature will accelerate the decomposition of organic carbon and thus provide more RMC for methanogenesis and produce more methane. Although the activity of methanotrophic bacteria may have significant increase due to the high temperature, more methane may be emitted if methane production is higher.

Some improvements should be made in future sample collection. The growth stage of plants, plant density and the amount of plant in each
sample should be as equal as possible. To understand the fate of the TNC that is supposed to go to grain filling in normal plants, information on the numbers of old and new tillers should also be collected.

Direct monitoring of the total organic carbon and the RMC in the soil is of great importance in understanding the variation of root exudates in rice field and can provide direct evidence on the loss of nonstructural carbohydrates due to the wounds of the rice plants. Further research on the relationship between the methane emission from rice paddies and the carbohydrate mobilization in rice plants could provide more information to understand the nature of the methanogenic process in rice fields and to evaluate attempts at methane mitigation through manipulating rice cultivation management and choosing proper rice cultivars.

4.7. Summary and Conclusions

In this thesis, a dynamic process of the nonstructural carbohydrates in rice plants is presented based on one growing season's observations. The changes of this dynamic process due to panicle cuttings are also reported. It is suggested that differing amounts of nonstructural carbohydrates of rice plants might be lost during the period of the season after plant cutting due to the stresses of root cut or panicle removal. Relative to the CH4 flux from the area of control plants, significant increases in methane emission were observed in each root cut or panicle cut treatments. Based on the analysis of the process from simple carbohydrate to methane emission (Sass
et al., 1991b), the amount of lost TNC corresponding to the increased methane emission is calculated. Also, the TNC loss is estimated through comparing the changes of TNC partitioning due to the plant manipulation. The TNC losses from these two calculations show a positive correlation, which suggests that due to the experimental plant manipulation, rice plants lose more nonstructural carbohydrates to soil than they normally do and, the lost carbohydrate resulted in methane, at least partially, through the methanogenesis.
V. REFERENCES


