

1-1-2011

Feedbacks of Methane and Nitrous Oxide Emissions from Rice Agriculture

Alec Sithole
Portland State University

Let us know how access to this document benefits you.

Follow this and additional works at: http://pdxscholar.library.pdx.edu/open_access_etds

Recommended Citation

Sithole, Alec, "Feedbacks of Methane and Nitrous Oxide Emissions from Rice Agriculture" (2011). *Dissertations and Theses*. Paper 43.

[10.15760/etd.43](https://doi.org/10.15760/etd.43)

This Dissertation is brought to you for free and open access. It has been accepted for inclusion in Dissertations and Theses by an authorized administrator of PDXScholar. For more information, please contact pdxscholar@pdx.edu.

Feedbacks of Methane and Nitrous Oxide Emissions from Rice Agriculture

by

Alec Sithole

A dissertation submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Applied Physics

Dissertation Committee:
M. Aslam K. Khalil, Chair
Anna -L. Reysenbach
Todd N. Rosenstiel
Mark D. Sytsma
Andrew L. Rice
Erik Bodegom

Portland State University
©2011

Abstract

The effect of global warming on methane (CH₄) and nitrous oxide (N₂O) emissions from agriculture was investigated and simulated from a soil warming experiment. Experiments were designed and installed in a temperature controlled greenhouse. The relationships between elevated temperatures and CH₄ and N₂O emissions were determined and calculated as the Q₁₀s of production, emission and oxidation. A study of the populations of methanogens and methanotrophs at a range of soil temperatures was performed based on soil molecular DNA analysis.

This study showed that global warming would increase CH₄ emissions from rice agriculture and that the resultant emissions will be potentially large enough to cause changes in the present atmospheric concentrations. This research also showed that this increase was most evident for soil temperatures below 30°C, above which emissions decreased with increasing temperature. The seasonal average Q₁₀s of CH₄ emission, production, oxidation, methanogen and methanotroph populations were found to be 1.7, 2.6 and 2.2, 2.6 and 3.8, respectively, over a temperature of 20-32 °C. Considering that the processes of CH₄ production and emission are similar to those in natural wetlands, which is the largest source of atmospheric CH₄, the contribution of this feedback is likely to cause a significant increase to the present CH₄ atmospheric budget if the current global warming trend persists over the next century.

The Q_{10} s of N_2O emissions and production were 0.5-3.3 and 0.4-2.9, respectively. The low Q_{10} values found for N_2O suggest that although global warming will have a direct impact on the production and emission rates. Nevertheless, the magnitude of the impact of global on both CH_4 and N_2O emissions from agriculture is likely to vary from one region to another due to the spatial variations in agricultural soil temperatures and the likely changes in the global regional distribution of water resources (water tables, rainfall patterns), water management practices and the responses of terrestrial CH_4 and N_2O sources such as natural wetlands and plants.

Acknowledgements

Today, this incredible and monumental journey has come to an end, but with a prize. This prize has come largely as a result of the unwavering guidance from my supervisors, moral support from colleagues and family members, and all those who proof read my work.

First and foremost, this research would not have been completed without financial support from my doctoral advisor Professor Aslam Khalil through his grant from the Office of Science (BER), U.S. Department of Energy grant (DE-FG02-08ER64515) during my entire graduate studies at Portland State University. He was very instrumental in defining the direction of this research, and was always available and open to discuss new ideas and answer my pertinent questions. I am also indebted to Dr. Andrew L. Rice for his guidance on designing isotope experiments, making his Continuous-flow Gas Chromatography-Isotope Ratio Mass Spectrometer available for running these samples, and for discussions of the results. From our research group, I am very grateful to Martha J. Shearer who helped me in the purchasing of material and setting-up my experiments. Special thanks also go Dr. Christopher Butenhoff who took a great task of proof reading my draft papers and dissertation.

My profound gratitude also goes to Professor Anna-Louise Reysenbach for her wealth of advice, good ideas and directives on running molecular DNA experiments, being available to discuss my experiments and for giving me full access to her lab instruments

and material that were funded through her grant from the U.S. National Science Foundation (OCE-0728391). I also learned a lot from her expertise and constructive criticism. In this work, I got great help from Gilbert Flores. He taught me protocols for soil molecular DNA extraction and the use of qPCR techniques in quantifying methanogens and methanotrophs, and how to interpret the results. Gilbert was very patient and available to assist me despite his tight schedule.

The enormous tasks involved in this research could not have been finished in time without the help of Austin Fischer and Erica Hanson who helped me with running of flux and isotope samples, respectively.

Lastly, I would like to thank my family for their encouragement and moral support during my studies.

Table of Contents

Abstract.....	i
Acknowledgements.....	iii
List of Figures.....	ix
List of Tables	xviii
Chapter 1	1
Background Information.....	1
1.1 Introduction.....	1
1.2 Q ₁₀ of CH ₄ and N ₂ O.....	7
1.3 Research Objectives.....	8
1.4 Scope and General Outline	10
Chapter 2.....	12
Experimental Design, Construction and Validation	12
2.0 Summary.....	12
2.1 Introduction.....	13
2.2 Soil Heating System.....	14
2.3 Temperature Measurements.....	18
2.4 Water Bath Temperature Stability	20
2.5 Tub and Flux Chamber Design.....	21
2.6 Leakage Tests.....	23
2.7 Conclusion	25
Chapter 3.....	26
Temperature Limits and Dynamics of Methane Fluxes from Rice Agriculture	26
3.0 Summary.....	26
3.1 Introduction.....	26
3.2 Aim and Objectives.....	29
3.3 Materials and Methods.....	29
3.3.1 Sample Collection.....	30
3.3.2 Data Analysis	31
3.4 Results and Discussion	33

3.4.1	Seasonal Flux Variations	33
3.4.2	Q ₁₀ of CH ₄ Fluxes	36
3.5	Conclusion	40
Chapter 4	41
Dynamics of CH ₄ Concentration in Pore Water under Elevated Soil Temperature		41
4.0	Summary	41
4.1	Introduction.....	42
4.2	Aim and Objectives.....	43
4.3	Materials and Methods.....	44
4.4	Results and Discussion	47
4.5	Conclusion	60
Chapter 5	61
Temperature Dependence of Methane Production		61
5.0	Summary	61
5.1	Introduction.....	61
5.2	Aim and Objectives.....	64
5.3	Material and Methods	65
5.4	Results and Discussion	67
5.5	Conclusion	72
Chapter 6	74
Quantifying Temperature Effect on Methane Oxidation in Rice Ecosystems using Stable Carbon and Hydrogen Isotope Ratios		74
6.0	Summary	74
6.1	Introduction.....	75
6.1.1	Other Factors Affecting CH ₄ Oxidation in Flooded Soils	77
6.1.2	Quantifying CH ₄ oxidation	78
6.1.4	Quantification of CH ₄ Oxidation	82
6.1.5	Isotope Fractionation by Transport.....	85
6.2	Aim and Objectives.....	87
6.3	Materials and Methods.....	87

6.3.1	Site Characteristics.....	87
6.3.2	Sampling Techniques.....	88
6.3.3	Isotopic Measurements	89
6.3.4	Determination of Fraction of CH ₄ Oxidized.....	90
6.4	Results and Discussion	90
6.4.1	Carbon Isotopic Composition	90
6.4.2	Relationships between $\delta^{13}\text{C}$ and δD in Emitted and Dissolved CH ₄	96
6.4.4	Fraction of CH ₄ Oxidized	99
6.5	Conclusion	100
Chapter 7.....		102
Response of <i>mcrA</i> and <i>pmoA</i> Gene Copies and Methane Fluxes		102
to Soil Temperature Changes in Rice Microcosms.....		102
7.0	Summary	102
7.1	Introduction.....	103
7.2	Aims and Objectives	105
7.3	Materials and Methods.....	106
7.3.1	Preparation and Collection of Soil Samples	106
7.3.2	Soil Microbial DNA Extraction, Quantitation and Purity	107
7.3.3	qPCR Detection of Methanogens and Methanotrophs.....	108
7.3	Statistical Analysis.....	109
7.5	Results and Discussion	110
7.5.1	DNA Purity and Yield.	110
7.5.2	Abundance of <i>mcrA</i> Genes	113
7.5.3	Abundance of <i>pmoA</i> Genes.....	115
7.5.4	Relative Abundance between <i>mcrA</i> and <i>pmoA</i> Genes.....	117
7.5.5	Regression between <i>pmoA</i> , <i>mcrA</i> Copies and Temperature	120
7.5.6	Relationships between CH ₄ Fluxes and <i>mcrA</i> and <i>pmoA</i> Copies	123
7.5.7	Q ₁₀ of Methanogens and Methanotrophs	125
7.6	Conclusion	127
Chapter 8.....		129

Nitrous Oxide Emissions from Rice Agriculture under Elevated Soil Temperatures ..	129
8.0 Summary	129
8.1 Introduction.....	130
8.2 Aim and Objectives.....	133
8.3 Materials and Methods.....	133
8.4 Results and Discussion	134
8.4.1 N ₂ O Fluxes.....	134
8.4.2 Effect of Soil Temperature on Fluxes.....	136
8.4.3 Effect of Temperature on N ₂ O Production Rates	139
8.4.3 Pore Water Concentrations	140
8.5 Conclusion	143
Chapter 9.....	144
Summary and General Conclusion	144
References.....	148
Appendix A.....	164
Seasonal average temperature data	164
Appendix B	165
Average CH ₄ and N ₂ O Flux Data	165
Appendix C	169
Average CH ₄ and N ₂ O Production Data	169
Appendix D.....	170
Pore Water CH ₄ and N ₂ O concentrations	170
Appendix E	172
Average CH ₄ Isotope Measurements	172

List of Figures

- Fig. 1.1: The combined radiative forcing estimates of CH₄ and N₂O is about 40 % that of CO₂. 2
- Fig. 1.2: Estimates of CH₄ from rice paddies as a percentage of the global annual total. Data from Miller (2004). 3
- Fig. 1.3: Three major mechanisms involved in CH₄ emission from rice paddies are production, oxidation and transport. Modified diagram adapted from Neue and Roger (1993). 4
- Fig. 1.4: Estimates of N₂O emissions from agriculture as a percentage of total anthropogenic emissions. Data from EDGAR, 1995. 5
- Fig. 2.2: Water bath and heating tanks were thermally insulated using fiberglass material. The top of water bath containers covered with Thermasheath 3 Insulation material to reduce heat loss. 17
- Fig. 2.3: Set-up with digital Immersion heater/circulator (enlarged) connected to the heating reservoir, showing the main components of the heating system: heater, circulator and a temperature sensor (PolyScience Inc). The immersion heaters had inbuilt circulators and thermostats that would help to control the water temperatures. The circulation speed was set to 15 liters/minute and temperatures were set at either, 20, 26, 30 or 39 °C. Flow valves were installed onto the water circulation system of each of the four heating systems to control the flow rate and allow mixing of warm water inside the water bath tanks. 18
- Fig. 2.4: Schematic of the temperature measurement unit for the water bath. Components labeled TH1-TH12 are Type-T thermocouples (solid red lines) that

were fitted to the production zone (~10cm depth) in both planted and unplanted tubs. The thermocouples labeled TH9-12 (blue) were connected to production experiment jars (solid blue lines and circles)..... 19

Fig. 2.5: Microloggers connected to the thermocouples and covered (inside a reflective cover) to protect them against adverse greenhouse conditions such as wetting and direct heating. The microloggers were programmed to measure temperatures every 5 and 30 minute intervals. To determine CH₄ and N₂O concentrations, pore water samplers (Khalil et al., 2008) were embedded into the soil in both planted and unplanted tubs. 20

Fig. 2.7: Acrylic flux chambers fitted with 12VDC fans to homogenize the air inside the flux during sampling, and a septum through which the gas samples were drawn (design based on Khalil et al., 1991). The edges were joined together using a hot glue gun. Thin PVC strips were glued from the bottom of the chamber to strengthen the chamber and prevent any gas leakage during sampling. A rubber septum was installed at the top of each flux chamber and was used to draw gas samples for CH₄ and N₂O flux measurements. 22

Fig. 2.8: A schematic representation of the components of production experimental set-up. The glass jars were made from modified Erlenmeyer flasks (necks extended by 20 cm). Inside the jars were 5 mL cylinders (cut from 30 ml plastic syringes) held by a very thin and in extensible string. Air-tight inlet and outlet valves, and insulated thermocouple temperature probes were fitted onto the stoppers and glued using a hot glue gun. 23

Fig. 3.1: (a) Rice field fluxes from Tu Zu China (reported in Khalil et al., 1998a), and (b) New Delhi, India (Parasher et al., 1993). Parashar et al. (1993) reported a distinct increase in CH₄ emission from rice plots with increase in soil temperature from 26 to 34.5 °C and a decrease in the rate of emission above the temperature under controlled soil temperature. Similar results can be observed in results reported by Khalil et al. (1991). All data indicate a sharp decline in CH₄ fluxes after reaching an optimum value or peak, an indication that the Arrhenius model will not consistently hold for temperature above 30 °C..... 28

Fig. 3.3: Seasonal CH₄ fluxes across different temperature treatments and between winter and summer seasons. 34

Fig. 3.4: Q₁₀ of CH₄ fluxes for growing seasons. 37

Fig. 3.5: Uncertainties in the Q₁₀ of CH₄ fluxes during the growing season..... 38

Fig. 3.6: Q₁₀ of CH₄ fluxes during the growing season, showing high values of Q₁₀ for low flux values..... 39

Fig. 4.1: Schematic diagram of pore water samplers (Khalil et al., 2008) inside the tubs. The samplers were inserted at 5, 10, 15 and 20 cm soil depths in each of the tubs. The cylinders were covered with gauze fabric to allow water movement between their interior and the surroundings..... 44

Fig. 4.2: Pictures of pore water tubes embedded into flooded soils inside tubs containing temperature probes. The tubes were fitted with valves to which the syringes were hooked during sampling..... 45

Fig. 4.3: Temperature effect on the seasonal average CH₄ concentration vertical profiles in the soil water in for planted tubs. In all four different temperature treatments, the average values are close to each other, with highest values at 20 cm depth..... 48

Fig. 4.4: Vertical pore water CH₄ concentrations profiles for 0-60 DAT, indicating layering in different temperature treatments. In each treatment, highest values occur between 10 and 15 cm. Differences in the profiles shows that temperature is an important factor during this phase of the season..... 50

Fig. 4.5: Effect of temperature on CH₄ concentration in pore water, showing higher concentrations at 32 °C at the beginning and lower at the end of the growing season. Lowest concentrations were present at lowest temperatures. Concentrations increase at the start of the growing season, reaching a peak at mid-season, and decrease as the season ended. 54

Fig. 4.6: Average concentration of CH₄ is higher at 32 °C at the start and lower at the end than at other temperatures. Concentrations were low at 20 °C throughout the season..... 55

Fig. 4.7: Average concentration per temperature set-up. Effect of transport and temperature on pore water CH₄ concentrations. Generally higher concentrations of CH₄ in control tubs at the beginning of the season, but lower at the end. These observations could be due to bacteria degrading decaying roots..... 56

Fig. 4.8: Trends of pore water CH₄ concentrations across different temperatures. Concentration of CH₄ at 32 °C is higher till mid-season, but lower than that at 28 °C thereafter. This is consistent with flux observations, in which fluxes have been

found to be higher at for 32 °C at the start of season, but quickly diminished after mid-season, with concentration at 28 °C becoming higher beyond this point..... 57

Fig. 4.9: Between 1 and DAT 60, pore water CH₄ concentration was the limiting factor in CH₄ fluxes. During this period fluxes increased to a maximum. In all treatments, CH₄ concentrations were highest and lowest at 32 °C and 20 °C..... 58

Fig. 4.11: CH₄ fluxes reaches maximum (25-40 mg/m²/hr) at 60 DAT (while the pore water concentrations increased from 60 µg/L to 1000 µg/L), and then level off despite continuous increase in CH₄ pore water concentration. 59

Fig. 5.1: Effect of temperature on the percentage contribution of acetate and H₂/CO₂ to methanogenesis in anoxic rice soil. The data shows that as temperature was continuously increased above 35 °C, CH₄ production contribution from H₂/CO₂ became predominant, while that from acetate diminished. Data extracted from Conrad (2002). 63

Fig. 5.2: Picture of production jars mounted onto the water bath system. The jars are fitted with valves and thermocouples connected to microloggers (not shown in picture). Temperature was continuously monitored and recorded every 5 minutes during the growing season. The glass jars were purged with N₂ several times before each sample was collected. 66

Fig. 5.3: CH₄ production rates, showing increases after 2 weeks following flooding. The highest production rates were associated with highest temperature after 17 days following transplanting (DAT). 68

Fig. 5.4: (a) Average CH₄ production rates as a function of temperature, and (b) Q_{10s} of CH₄ production based on the incubation technique..... 70

Fig. 5.5: CH ₄ oxidation rates and fractions calculated from production and flux data...	72
Fig. 6.1: Typical models proposed for the variation of α_{ox} with temperature. There is generally an agreement between the slopes, but with significantly different intercepts.	85
Fig. 6.2: Seasonal variation of $\delta^{13}C$ of CH ₄ emitted from rice planted tubs (solid lines) and unplanted tubs (broken lines) for different temperature treatments. In all treatments, the samples from planted tubs were more depleted during the middle part of the growing season than at the start. That feature was not evident in unplanted (control), except in one particular treatment (20 °C) in which the trend was similar to those in planted tubs.	91
Fig. 6.3: Seasonal variation of $\delta^{13}C$ of CH ₄ in pore water from planted tubs (solid lines) and unplanted tubs (broken lines) for different temperature treatments.	92
Fig. 6.4: Time series of δD of CH ₄ showing hydrogen isotopic composition in rice planted tubs (solid lines) and unplanted (control) tubs (broken lines) for different temperature treatments. Generally, the value are enriched at the start, and then depleted till the end of sampling period.	93
Fig. 6.5: Time series of pore water δD of CH ₄ showing hydrogen isotopic composition in rice planted tubs (solid lines) and unplanted (control) tubs (broken lines) for different temperature treatments.	94
Fig. 6.6: Classification of bacterial and thermogenic natural gas by the combination of $\delta^{13}C-CH_4$ and $\delta D-CH_4$ information (Adapted from Whitcar 1999). Compared with $\delta^{13}C-CH_4$ and $\delta D-CH_4$ found in this research, results showed that the predominant source of CH ₄ was acetate (red circle), and not through CO ₂ reduction.	95

Fig. 6.7: Effect of temperature on the relationship between isotopic composition, $\delta^{13}\text{C}$ and δD values in the air samples from planted tubs. 97

Fig. 6.8: Temperature effect on the relationship between isotopic composition, $\delta^{13}\text{C}$ and δD values in pore water from planted tubs. 98

Fig.7.1: Schematic of the replicates (Rice planted tubs) and tubs from which the soil samples for population studies will be taken (rice-planted sampling tubs), and controls for each set of experiment (unplanted/Control tubs). 107

Fig. 7.2: Protocol for the qPCR thermal profile set-up for *mcrA* gene quantitation were as follows: 95 °C for 15 min, 40 cycles of 94 °C for 30s, 56 °C for 30s, 72 °C for 1 min, followed by a single cycle of 82 °C for 10s..... 109

Fig. 7.3: Ratios of 260/230 (e.g. >2) and 260/280 (e.g. >1.7) are indicative of pure DNA, while the low ratios are indicative of humic acid and protein contamination, respectively. 111

Fig. 7.4: Total DNA yields ranged from of 10 to 45 μg of DNA per μl . Data showed no notable differences across different temperature treatments..... 112

Fig. 7.5: Average values of DNA concentration at different soil horizons at different soil temperature treatment..... 113

Fig. 7.6: Average values of *mcrA* copies at different soil horizons at different soil temperature treatment. Highest *mcrA* copies were found at 28 and 32 °C. 114

Fig. 7.7: Average values of *pmoA* copies at different soil horizons at different soil temperature treatment. Highest *pmoA* copies were found at 28 and 32 °C..... 116

Fig. 7.8: Methanogen and methanotroph populations at different soil horizons at different soil temperature treatments. 117

Fig. 7.9: Relative abundance of <i>mcrA</i> and <i>pmoA</i> (copies g ⁻¹ dry soil) at different depths	119
Fig. 7.10: Regressions: natural log (<i>pmoA</i> copies g ⁻¹ dry soil) versus natural log (<i>mcrA</i> copies g ⁻¹ dry soil).....	121
Fig. 7.11: CH ₄ fluxes vs <i>mcrA</i> a copies at 5-10 cm depth	124
Fig. 7.12: CH ₄ fluxes vs <i>pmoA</i> copies at 5-10 cm depth	125
Fig. 7.13: (a) Dependence of methanogen and methanotroph populations on soil temperature. (b) Q ₁₀ of methanogen and methanotrophs for different soil horizons.	126
Fig. 7.14: Variation of Q ₁₀ values of methanogens and methanotrophs during the season for different temperature treatments and soil depth.....	127
Fig. 8.1: Dominant processes involved in the formation of N ₂ O in soils: (I) Nitrification (oxidation of ammonium to nitrite and nitrate, or a biologically induced increase in the oxidation state of nitrogen and occurs under aerobic conditions) and (II) Denitrification (biological reduction of nitrate, NO ₃ ⁻ or nitrite, NO ₂ ⁻ , to nitrogen oxides or molecular nitrogen under anaerobic conditions). Adapted from Sithole (2009).....	131
Fig. 8.2: N ₂ O emissions from both (a) planted and (b) unplanted tubs. Higher fluxes occurred in unplanted than in planted tubs, and the peaks coincide with episodes of fertilization. Elsewhere, the fluxes were nearly close to zero or slight negative. The impact of temperature was more apparent in unplanted tubs than in planted ones.	135

Fig. 8.3: Effect of soil temperature on N₂O fluxes during (a) Winter and (b) Summer.
..... 137

Fig. 8.3: Effect of soil temperature on fluxes as indicated by Q₁₀ values. No discrepancy
was observed between the data from (a) Winter and (b) Summer..... 138

Fig. 8.5: Average pore water concentrations of N₂O with increasing soil temperature in
the rhizosphere. The differences could be due to plant transport (increased
transport through rice) and N utilization by plants. 142

List of Tables

Table 1.1: Reported Q_{10} values.....	8
Table 2.1: The t-statistic values and slope (ppm/minute) of regression between measured concentrations for: (a) CH_4 , and (b) N_2O . H_0 was rejected for $t_{\alpha=0.025} \leq -3.184$ and $t_{\alpha=0.025} \geq +3.184$ for $n = 5$ (two-tailed test).....	24
Table 6.1: Overview of typical isotope measurements on CH_4 from rice paddies.....	79
Table 6.2: Overview of the kinetic isotopic effects during CH_4 oxidation by anaerobic bacteria.....	82

Chapter 1

Background Information

1.1 Introduction

One of the major environmental threats our planet faces today is the potential for rapid global warming. Determining the potential causes of global climate change has been a long term process that has involved work from scientists around the globe. From more than three decades of scientific studies, questions have been raised about the magnitude of the projected global warming, its environmental impacts, and the role of feedbacks between greenhouse gases and elevated global temperatures over the next century.

It is noteworthy that the combined global warming contribution from these non-CO₂ greenhouse gases is quite substantial. For example, among non-CO₂ greenhouse gases, methane (CH₄) has the highest radiative forcing, contributing about 3.3 GtCO₂-eq/yr (IPCC, 2007d). At its present atmospheric concentration of 1775 ppb, which is about three times its pre-industrial level (Rasmussen and Khalil, 1981; Khalil et al., 1989), it has an estimated radiative forcing (RF) of $+0.48 \pm 0.05 \text{ Wm}^{-2}$ (IPCC, 2007b), nearly one third the contribution of CO₂ (Fig 1.1).

The predominant source of CH₄ is microbial activities in anaerobic environments such as gastrointestinal tracts of ruminants, rice paddies, landfills and natural wetlands (Khalil and Shearer, 1993a; Khalil and Shearer, 1993b; Bodelier et al., 2000).

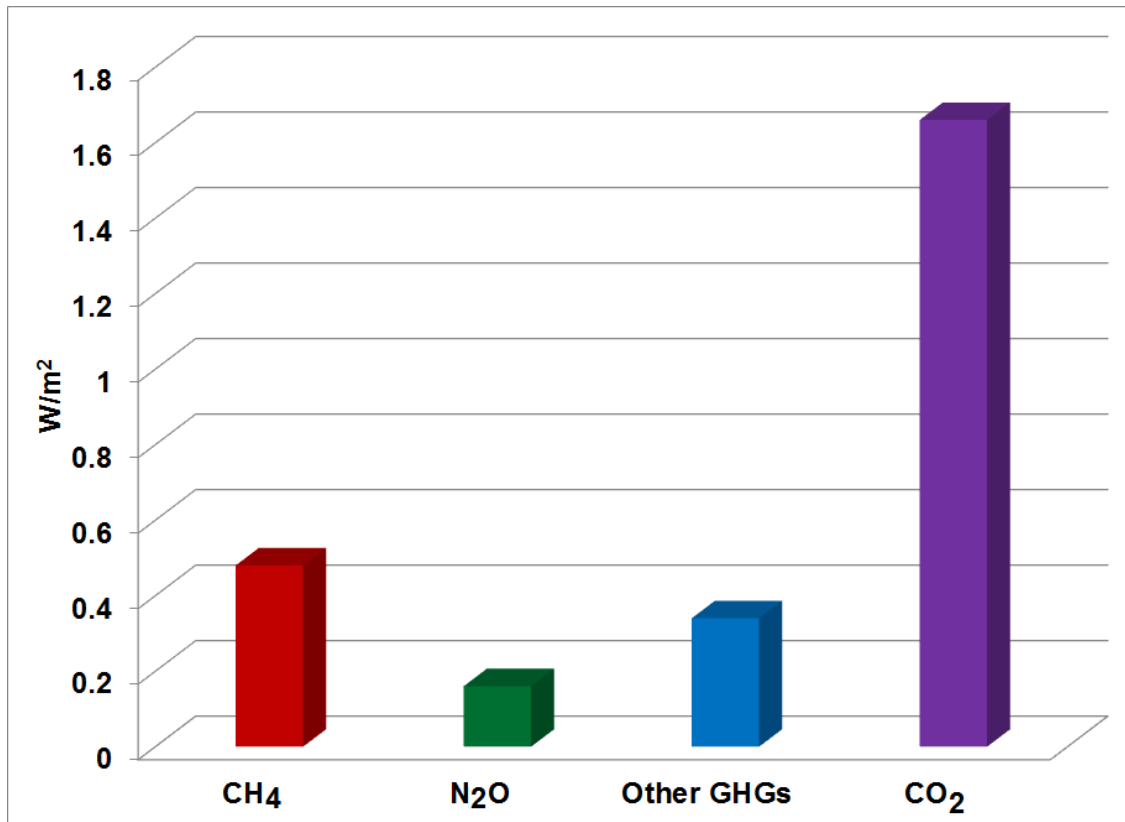


Fig. 1.1: The combined radiative forcing estimates of CH₄ and N₂O is about 40 % that of CO₂.

While anthropogenic sources account for about 70 % of global annual total CH₄ emissions (Allen et al., 2003; IPCC, 2007a), about 40-60 % of this contribution comes from agriculture (Zou et al., 2004; Minami, 1997; Whiting and Chanton, 1993). On a global scale rice fields contribute an estimated 14 % total global CH₄ emission (Fig. 1.2).

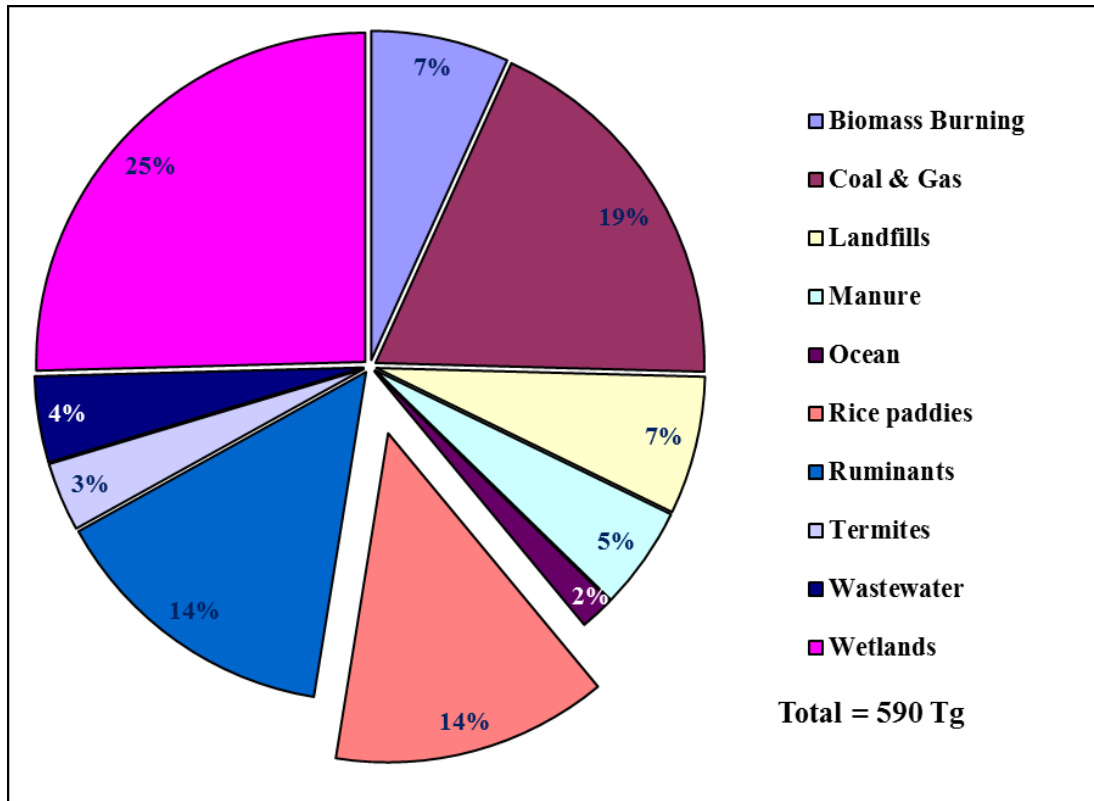


Fig. 1.2: Estimates of CH₄ from rice paddies as a percentage of the global annual total. Data from Miller (2004).

The source of CH₄ production substrates is decaying organic material by microorganisms. One group of these microorganisms (methanogens) produces CH₄ by utilizing acetate or through CO₂ reduction, while the other group (methanotrophs) oxidizes some of the CH₄. The production and oxidation of CH₄ are exclusively anaerobic and aerobic processes, respectively. The CH₄ that is not oxidized is transported by physical processes such as diffusion, ebullition and ventilation. Ventilation through the aerenchyma is the major transport process rice paddies, while the other transport processes are predominantly active during the first two weeks before crop establishment after transplanting.

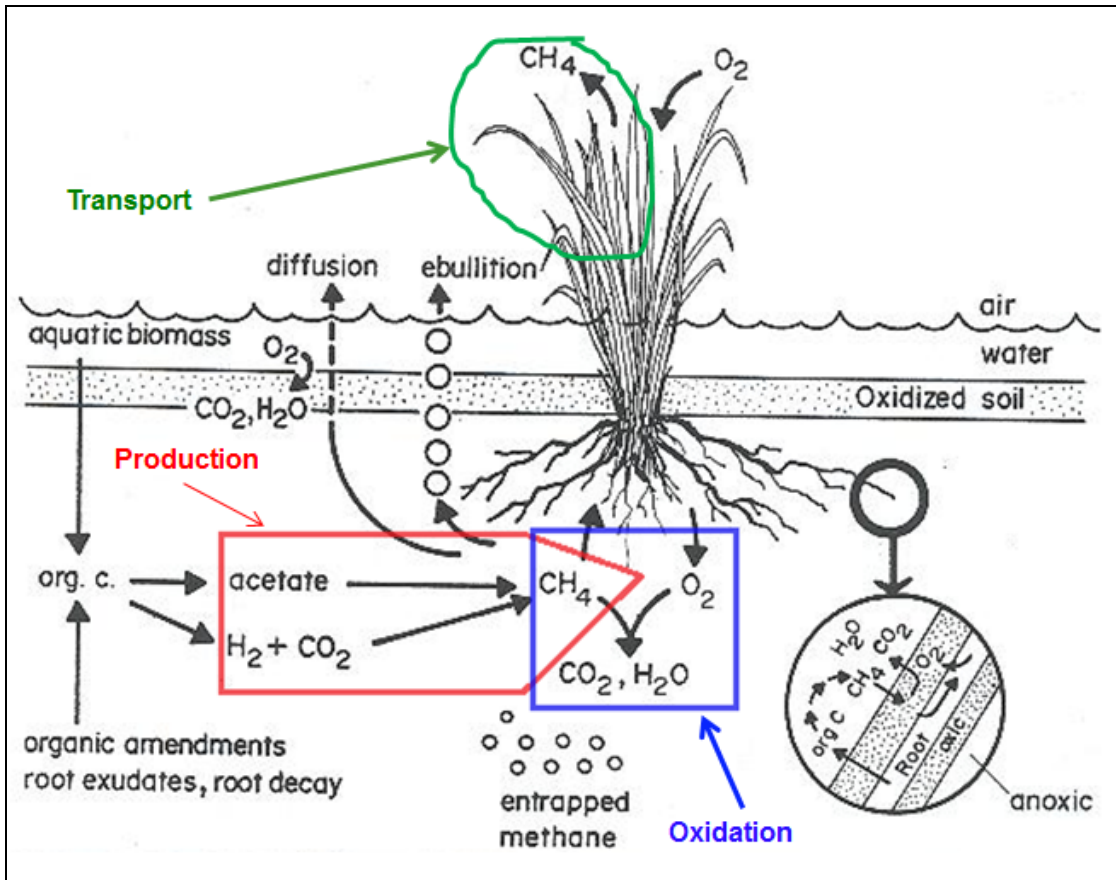


Fig. 1.3: Three major mechanisms involved in CH₄ emission from rice paddies are production, oxidation and transport. Modified diagram adapted from Neue and Roger (1993).

Another important greenhouse gas, N₂O, is about 300 times more effective in global warming than CO₂ per molecule (Minami 1997). At its current level of about 319 ppbv (Beerling et al. 2007), which represents an increase of 18 % from its pre-industrial level of about 270 ± 7 ppbv (Forster et al. 2007), N₂O has a GWP of 2.8 Gt CO₂- eq/yr (Smith *et al.* 2007), and is mainly produced from agricultural soils (Fig.1.4).

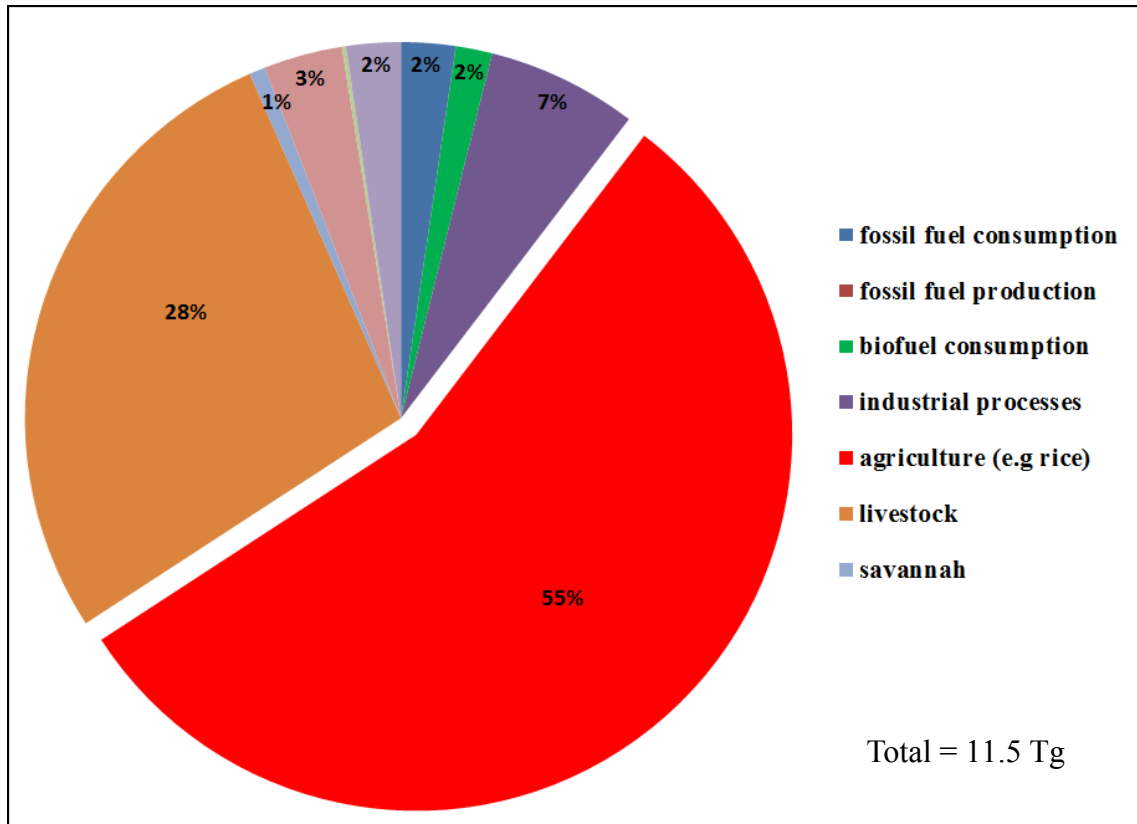


Fig. 1.4: Estimates of N₂O emissions from agriculture as a percentage of total anthropogenic emissions. Data from EDGAR, 1995.

Ranked according to contribution to global warming so far, CH₄ and N₂O are considered second and third respectively after CO₂.

While it is clear from field and laboratory studies that higher soil temperatures affect CH₄ and N₂O emissions (Tab. 1.1), quantitative answers as to how global warming will affect these emissions are presently not well known. Considering that rice paddies are similar to wetlands, with a gross contribution of about 40-50 % of the global source, the contribution of this feedback to global warming is likely to be quite substantial. Undoubtedly, this feedback between global warming and these greenhouse gases will be

an important component of the future climate change. While the atmospheric concentration of gases is critically dependent upon the long-term sensitivity of these emissions to global warming, the lack of precise information about this sensitivity means that climate models may underestimate climate change over the next century. Thus, there is need to distinguish the different processes that lead to CH₄ and N₂O emissions, their temperature dependency, and their response to global climate change. The ability to adequately address these issues is the key to understand these feedbacks, explain the observed trends, know what to manage and what to include in the strategy to control the future concentrations of these greenhouse gases.

Previous greenhouse results indicated increases of CH₄ and N₂O fluxes from flooded rice tubs in response to increased soil temperatures (Sithole, 2009). From these early experiments, more questions emerged, and the need for further investigations was highlighted: (I) how will an increase in soil temperature affect the production, oxidation and transport of these gases, and subsequently the Q₁₀ of the net emissions? (II) What is the linkage between the Q₁₀ of plant-mediated transport and the Q_{10s} of production and oxidation? The need to develop the theory and relationships between these Q_{10s} was stressed in order to understand the complexity of the response of these processes to any change in temperature.

The IPCC projections have indicated that global temperature may rise by between 1.4 and 6 °C by 2100 (depending on the scenario). Because of large variations in data reported from different studies, one of the thorny issues is to unify these results and use

the data to project future emissions. It is important to note that this feedback should be incorporated into climate model to improve the predictability of future CH₄ and N₂O emissions from rice paddies and similar ecosystems such as natural wetlands. For this purpose, it is necessary to quantify this feedback in order to understand whether global warming will promote any preferential enhancement of either sources or sinks.

1.2 Q₁₀ of CH₄ and N₂O

Already, several studies have shown that that CH₄ and N₂O fluxes increase with increasing soil temperature (Table 1.1). The large variation in the reported Q₁₀ values is clear testimony that temperature plays an important but poorly understood role in regulating these greenhouse gas emissions. Either non-biological processes may be influencing this response (Grant and Pattey, 2008) or the large Q₁₀ values could reflect the anomalous temperature behavior of microbial processes or their interactions in the underlying processes (Segers, 1998). For example, simultaneous changes in temperature and substrate availability (Whiting and Chanton, 1993) or changes in substrate availability coincident with temperature increase are being mistaken for a temperature response.

Table 1.1: Reported Q_{10} values.

Q_{10} values	Process	Method	Temperature (°C)	Source
1.27 - 2.25	CH ₄ oxidation	Incubation: drained peatland soil	5.5 - 15	Crill et al., 1994
3.0 - 3.3	Net CH ₄ emission	Static closed chambers: individual plants	5.0 - 30	MacDonald et al., 1998
1.1	CH ₄ oxidation	Incubation: slurried peat samples	4.0 - 13.5	Hulzen et al., 1999
1.5			13.5 - 30	
1.3 - 28	CH ₄ production	Incubation: slurried peat samples	-	Segers, 1998
1.2 - 2.0	CH ₄ oxidation	Incubation: peat soil from fertilized & unfertilized plots	-	Crill et al., 1991
1.7 - 4.7	CH ₄ production	Incubation: anaerobic soil from Northern Canadian wetlands	10 - 20	Valentine, 1994
3.1 - 8.7	CH ₄ production	Soil slurries measured using flux chambers.	2 - 24	Priemé, 1994
1.5 - 2.3	CH ₄ net emissions from rice fields	Static closed chambers: individual plants.	20 - 42	Khalil et al., 1998d
<1 - 6	CH ₄ emissions from flooded soils	Incubation flooded soil samples	15 - 25	Rath et al., 2002
>1 - 5			25 - 35	
16	CH ₄ emissions from flooded soils	Incubated paddy soil cores	20 - 25	Tsutsuki and Ponnampuruma, 1987
6	N ₂ O emission	Automated flux chamber measurements from potato field soils.	10 - 30	Flessa et al., 2002
50	N ₂ O emission	Incubated soil cores fertilized with ammonium nitrate.	5 - 50	Dobbie and Smith 2001
8.9			12 - 18	
3.7			5 - 50	
2.3		Incubated grassland soil cores	12 - 18	
2.2-7.7	CH ₄	Emission from rice ecosystems in the greenhouse	19 -23	Sithole, 2009
1.1-3.6	N ₂ O			

1.3 Research Objectives

Because of the complexity and gaps in the understanding of temperature dynamics of the processes involved in the formation and emission of these gases, the magnitude of

this feedback in the real earth system remain highly uncertain. From previous experiments, preliminary simplified experiments to measure Q_{10} of CH_4 and N_2O were carried out. From this scoping study, Q_{10} s for CH_4 and N_2O were found to be 2-6 and >1-7, respectively (Sithole, 2009). These results, although general, were informative and a compelling proof of concept. While increasing soil temperatures will increase emissions from rice agriculture, it is also important to note that other processes such as oxidation and transport may respond in similar manner, and these add the complexity to the present Q_{10} experiments and theory. Because there was so much variation in the Q_{10} s, a more detailed look at the processes from a mechanistic approach is therefore needed to understand the factors that caused this variation. Only then can a global estimate be made because the Q_{10} will not be the same for all places and under all conditions. It is important that these processes be isolated and their response to temperature be investigated since the final Q_{10} strongly depends on the Q_{10} s of the three processes above. For example, high Q_{10} values may be due to simultaneous changes in temperature and other environmental factors such as moisture content or substrate availability and/or changes in substrate availability coincident with temperature increase being mistaken for a temperature response.

The interaction between the different processes may result in widely different Q_{10} s of the fluxes from one place to another. This is analogous to the fact that CH_4 emissions are measured to vary from 1-2 $\text{mg}/\text{m}^2/\text{hr}$ for seasonal averages to 50 $\text{mg}/\text{m}^2/\text{hr}$ under normal field conditions. This is because of the interaction between the basic processes of production, oxidation and transport.

Just as it is not possible to extrapolate fluxes found in one set of greenhouse experiments to the world, the Q_{10} s cannot be extrapolated either. Instead the factors that control the Q_{10} are needed for extrapolation and upscaling. In the quest for answers to these questions, the feedbacks between global warming and two non- CO_2 greenhouse gases, CH_4 and N_2O , emitted from rice agriculture were simulated and investigated in this research. To achieve these objectives, this research was aimed at designing and developing methods to quantify the effect of temperature on CH_4 emission, production and oxidation from a mechanistic approach. The same experiments were used to investigate behavior of N_2O emissions and production under different temperature treatments under greenhouse conditions.

1.4 Scope and General Outline

In this work, the Q_{10} s were determined from a mechanistic point of view. Experiments were designed, constructed and tested as outlined in Chapter 2. The Q_{10} s of emission, oxidation and production were measured and results presented and discussed in Chapters 3, 4, and 5, respectively. Production rates were determined from standard incubation techniques, and oxidation fractions were determined from non-invasive natural stable carbon isotopic compositions. Concentrations of dissolved CH_4 in pore water were investigated and results are presented in Chapter 6. Considering that CH_4 is produced and oxidized in the rhizosphere by methanogens and methanotrophs, respectively, DNA and qPCR experiments were designed and performed to determine

the number of *mcrA* and *pmoA* copies in the rhizosphere. The *mcrA* and *pmoA* genes were the functional gene markers used to detect CH₄-producing *Archea* (methanogens) and CH₄-oxidizing bacteria (methanotrophs), respectively. The data are presented in Chapter 7. Experiments and results of N₂O emissions and production are presented in Chapter 8. A general summary of the key issues, the new discoveries, and the future research direction are presented in Chapter 9.

Chapter 2

Experimental Design, Construction and Validation

2.0 Summary

A controlled soil heating system for simulating global warming and its impact on greenhouse gas emissions from rice agriculture was developed and validated. The system consisted of 16 soil tubs whose temperatures were controlled by four water bath heating systems that were set-up in the research greenhouse at Portland State University, Oregon. This greenhouse environment was equipped with humidifiers, fans, automatic vents, HID fluorescent halide lamps and temperature control system. The system reported here supported studies of the Q_{10} of CH_4 flux, production and oxidation, and concentrations in pore water and microbial populations under four different elevated soil temperature regimes. Flux and production studies of N_2O were also studied under the same conditions.

Four different temperature treatments, with a 4 °C differential between them, were achieved through the use of four digital temperature control units. In order to achieve the correct environmental field conditions, the Wadsworth environmentally-controlled greenhouse ambient temperature was kept low by constraining it to between 18 and 24 °C whilst the daily “sunset” was maintained at 10:30 pm throughout the sampling period to match field conditions. Each of the four temperature treatments was thermally insulated to maximize the energy use of the system, and comprised a heating tank and the water bath container that housed four locally constructed tubs. To achieve the

required temperature range, the digital Immersion Circulator/Heater temperature set points were maintained at 20, 26, 32, and 39 °C, and the measured soil temperatures were 20, 24, 28 and 32 °C, respectively. These set points were maintained throughout the data acquisition period, with the exception of the two-week period between seasons during which the system maintenances were performed, a demonstration of the superior control of temperature as desired in these experiments. Highest heat losses occurred in the treatments that had the highest set point temperatures relative to ambient, where the set-point temperature was 39 °C, but the recorded soil temperature was 32 °C.

Tubs for holding soil and plants were constructed of PVC, flux chambers were constructed from Plexiglas sheets, and production glass containers were Erlenmeyer flasks with necks extended by 20cm. The tubs were tested for water leaks, and flux chambers and production containers were tested gas leaks before deployment. A statistical test (5 % significance level) to determine gas leakage on these glass containers indicated that the slopes of the regressions between the concentration and time were not significantly different from zero, a confirmation of the robustness of the production experiment apparatus over a 40-minute period for which CH₄ and N₂O production studies were performed on each treatment on a weekly basis.

2.1 Introduction

In previous experiments a heating pad at the base of the tub was used. Although these experiments produced a response of higher emissions with increasing temperatures,

there were a number of drawbacks. Most important were the fact that it was difficult to maintain constant temperatures and the heating of the soil was not uniform with warmest temperatures at the bottom while under natural conditions, warmer temperatures are expected at the top. Nevertheless, the experiments, although not precise, yielded a very important result that greenhouse gas emissions from agriculture, particularly, CH₄ indeed increased with elevated soil temperature. Equipped with these ideas, a robust system was designed and constructed to address problems outlined above, and ensure the correct quantification of effect of temperature on mechanisms that lead to the emissions of CH₄ and N₂O from rice agriculture.

2.2 Soil Heating System

Several water bath heating methods have been in use for several decades (Heninger and White, 1974; Borges and Chaney, 1989). Examples include the use of antifreeze to regulate temperatures or submerged pots in the heated water bath (Turner and Jarvis, 1975). In this research, the latter method was considered, but based on original ideas that were developed in this research. Temperatures of -2 °C, +2 °C, (mid-double CO₂ result) and +5 °C (IPCC scenario for 2100) relative to global average temperature were considered in the design of the water bath set-up. Expecting that beyond a certain critical temperature, flux would start dropping, a slightly higher temperature above the base (+10 °C) was chosen in order to catch the downturn point.

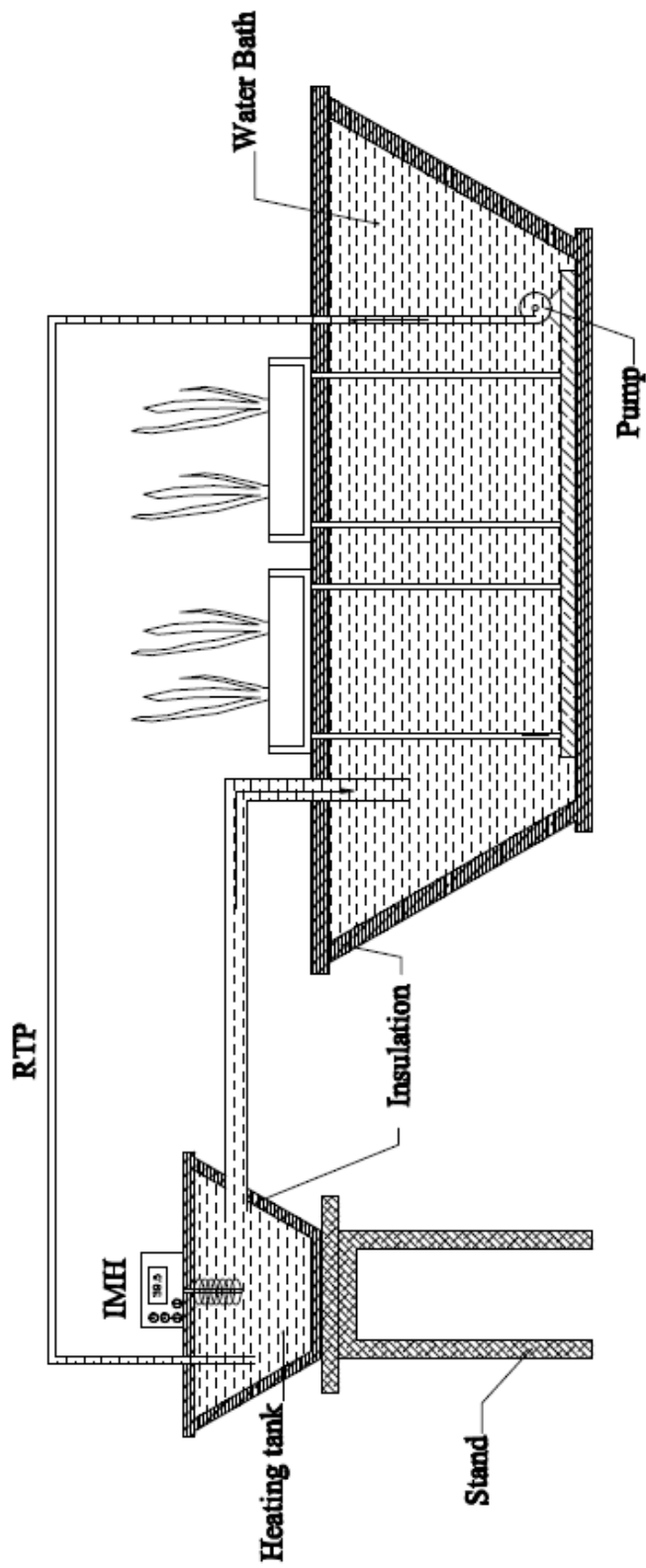


Fig. 2.1: A schematic side view of the water bath heating system. Water in the 50 gallon tank was pumped via the return pipe (RTP) into the heating tank in which an Immersion circulation heater (IMH) was connected. The heated water falls back into WB through gravitation. The Polyvinylchloride (PVC) tubes containing soil (labeled TB1 ... TB4) were directly immersed in a water bath.

The system was designed and constructed such that the relationship between soil temperature and the rates of processes that lead to CH₄ and N₂O emissions (namely: production, transport and consumption) could be quantified.

The designed temperature control system for soil heating experiments comprised four water heating systems and 16 tubs. The tubs were constructed using hardened PVC materials were mounted into the water bath heating systems as shown in Fig. 2.1. Each of the heating system consisted of two tanks: (I) a heating tank fitted with an Immersion Heater (IMH), and (II) a water bath container fitted with a water pump and four tubs. Inside the heating tank, the water was circulated through a spiral-shaped pipe connected to heating rods. To protect the Immersion Heaters (CIRC, 7306, IMM, 120/60, PolyScience Inc.) when the level of water fell below the recommended manufacturer's level, water was separately heated in the heating tank and channeled back into the water bath containing the tubs through gravity.

To preserve heat, the water was recycled by pumping back from the water bath to heating tank through RTP. The water flow rate was adjusted by setting the water pump flow rate to 15 liters/minute and through the pressure control valves to prevent overflow, and consequently unnecessary heat loss. To reduce heat loss, the tanks were insulated using a 10 cm thickness Fiberglass-R30 material and wrapped in Du Pont Tyvek Home Wrap. The top and base of WB covered with Thermasheath 3 Insulation (Fig. 2.2). Each WB comprised four tubs: one bare tub and three rice planted tubs. For each of four temperature treatments, the three rice planted tubs and one unplanted one each

contained temperature probes made in our laboratory using thermocouples positioned at the center of each tub. Seedlings were grown in potting soil and transplanted into the tubs after three weeks. The planting density and amount of fertilizer was similar to that applied under field conditions in China. This set-up allowed comparisons to be made between emissions from planted tubs and those from the unplanted tubs (control), and also within the same temperature treatment.



Fig. 2.2: Water bath and heating tanks were thermally insulated using fiberglass material. The top of water bath containers covered with Thermasheath 3 Insulation material to reduce heat loss.

Different temperatures were set to each of the four water heating systems by setting different set temperatures on immersion heaters as shown in Fig. 2.3.

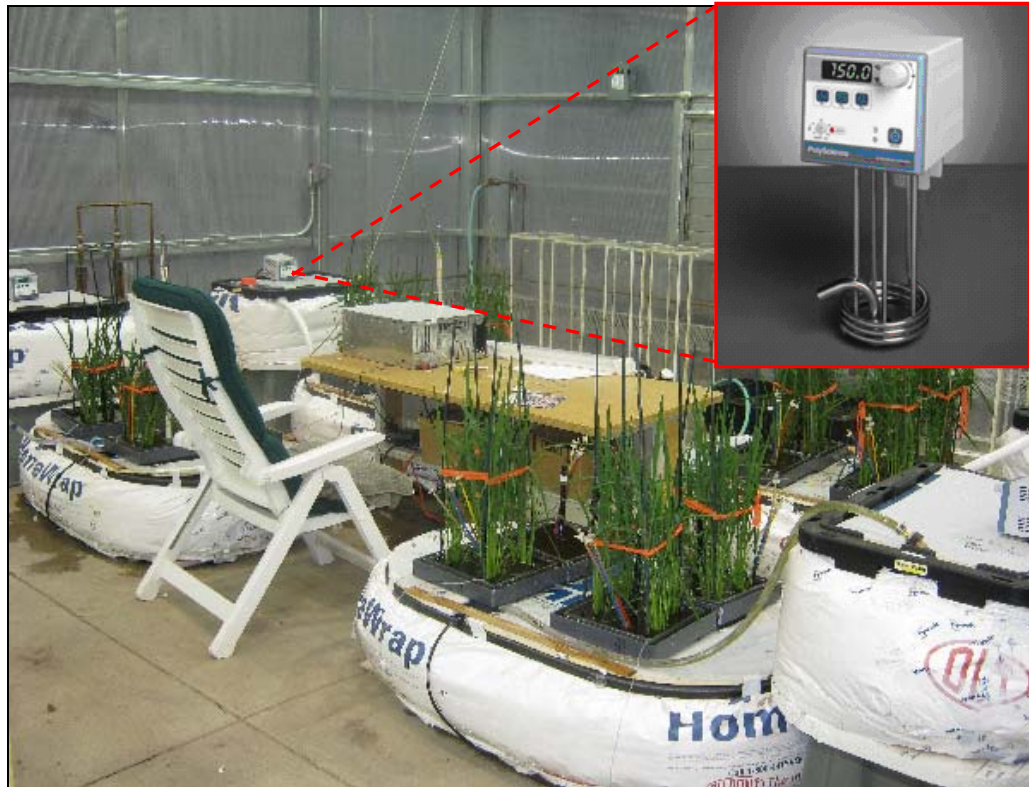


Fig. 2.3: Set-up with digital Immersion heater/circulator (enlarged) connected to the heating reservoir, showing the main components of the heating system: heater, circulator and a temperature sensor (PolyScience Inc). The immersion heaters had inbuilt circulators and thermostats that would help to control the water temperatures. The circulation speed was set to 15 liters/minute and temperatures were set at either, 20, 26, 30 or 39 °C. Flow valves were installed onto the water circulation system of each of the four heating systems to control the flow rate and allow mixing of warm water inside the water bath tanks.

2.3 Temperature Measurements

Two CR3000 microloggers (Campbell Scientific Inc.) were connected to Type T- Thermocouples (DigKey Inc.) inserted into the soil (at 10cm depth) in each of the tubs (Fig. 2.4 & 2.5). The microloggers were set to read, calculate and record soil temperature data every 5 seconds and average it over 5- and 30 minutes.

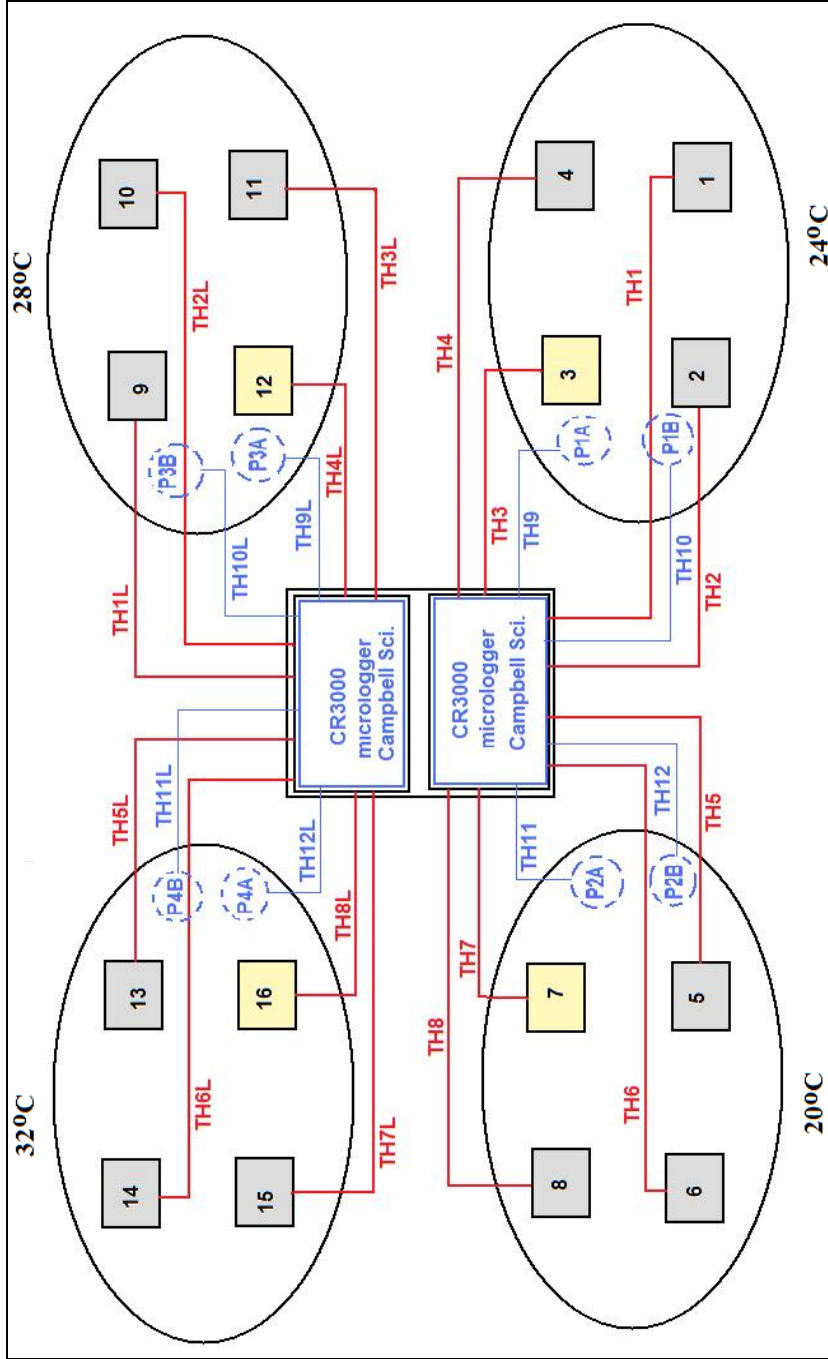


Fig. 2.4: Schematic of the temperature measurement unit for the water bath. Components labeled TH1-TH12 are Type-T thermocouples (solid red lines) that were fitted to the production zone (~10cm depth) in both planted and unplanted tubs. The thermocouples labeled TH9-12 (blue) were connected to production experiment jars (solid blue lines and circles).

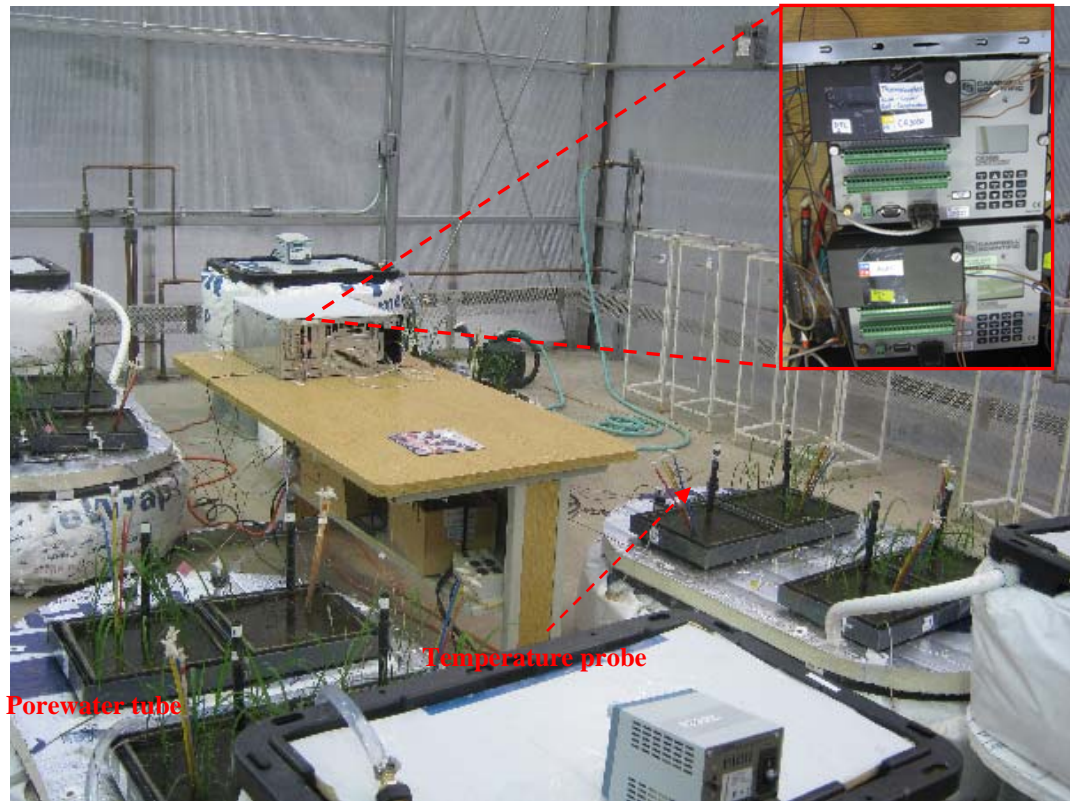


Fig. 2.5: Microloggers connected to the thermocouples and covered (inside a reflective cover) to protect them against adverse greenhouse conditions such as wetting and direct heating. The microloggers were programmed to measure temperatures every 5 and 30 minute intervals. To determine CH_4 and N_2O concentrations, pore water samplers (Khalil et al., 2008) were embedded into the soil in both planted and unplanted tubs.

2.4 Water Bath Temperature Stability

To determine the robustness of the system stability after installation, different parameters were monitored and evaluated for stability of the heat control system during the first two-week period and monthly thereafter. The temperature sampling interval was maintained at 5 minute intervals to capture any temperature disturbance during the 10-minute interval between flux measurements. Within each of the treatments, the soil temperatures were uniform across tubs, such that measurements from a single tub in

each set-up could provide a reliable estimate of the average soil temperature in each treatment. Analysis of temperature data during the first week showed that it took about one week for all the soil temperatures to reach thermal equilibrium with the water bath temperature. Thereafter, a time series analysis of the data revealed minimal changes in soil temperatures to changes in the ambient temperature (Fig. 2.6).

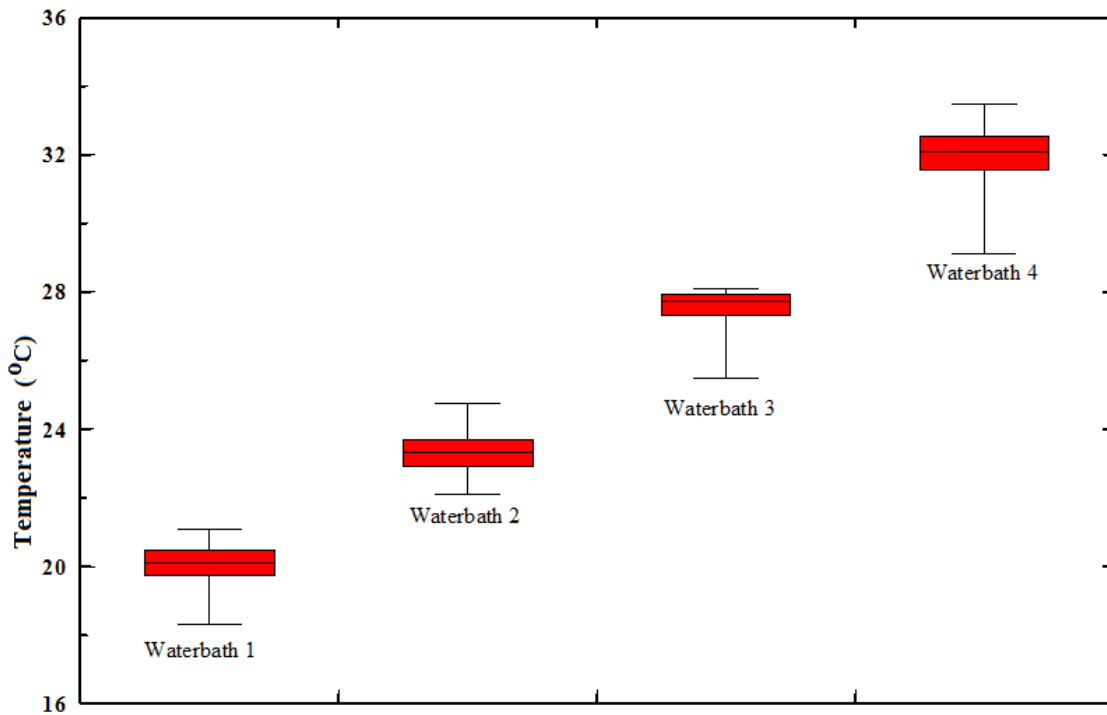


Fig. 2.6: Results of test for soil temperature stability over the first two weeks after installation of the experimental set-up. The data showed a steady state temperature differential of ~ 4 °C as desired. The box plots indicate the 1st and 3rd quartiles.

2.5 Tub and Flux Chamber Design

The tubs were constructed from (1/8 inch thickness) polyvinyl chloride (PVC) panels to allow four tubs to fit into each of the 50 gallon water bath containers. The gutters were

made from hollow rectangular PVC pipes and welded onto the tubs by using tuck-welding followed by a PVC welding machine, and eventually tested for leakage. The gutters were designed to fit the flux chambers and be flooded when the chambers were attached. This would create an air-tight seal between the chamber and the tub.

Flux chambers were constructed using Plexiglas and Blind Stop Vinyl materials. Fittings were achieved through the use of hot glue and brass screws. Fans were fitted to the top to homogenize the air inside the flux chamber.

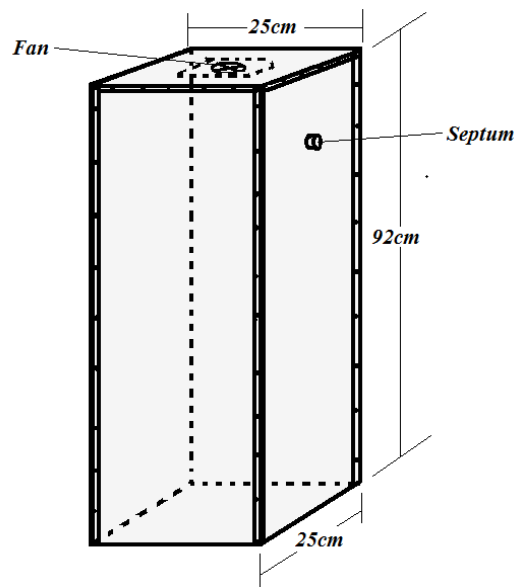


Fig. 2.7: Acrylic flux chambers fitted with 12VDC fans to homogenize the air inside the flux during sampling, and a septum through which the gas samples were drawn (design based on Khalil et al., 1991). The edges were joined together using a hot glue gun. Thin PVC strips were glued from the bottom of the chamber to strengthen the chamber and prevent any gas leakage during sampling. A rubber septum was installed at the top of each flux chamber and was used to draw gas samples for CH_4 and N_2O flux measurements.

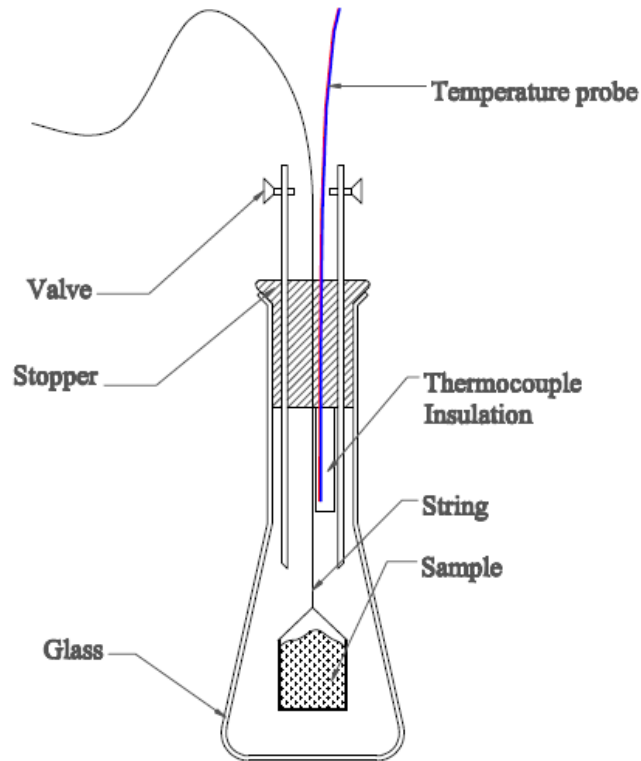


Fig. 2.8: A schematic representation of the components of production experimental set-up. The glass jars were made from modified Erlenmeyer flasks (necks extended by 20 cm). Inside the jars were 5 mL cylinders (cut from 30 ml plastic syringes) held by a very thin and in extensible string. Air-tight inlet and outlet valves, and insulated thermocouple temperature probes were fitted onto the stoppers and glued using a hot glue gun.

2.6 Leakage Tests

Before the set-up in Fig 2.7 was deployed into the greenhouse, the system was tested for CH₄ leakage. After a known dose of 1 % CH₄ was injected into each of the 10 jars, the valve was immediately closed and five air samples were collected at 10-minute intervals. The collected gas samples were measured for both CH₄ and N₂O by a gas chromatography with FID (flame ionization detector) and ECD (electron capture

detector). From the slope, b , regression of concentration and time, t statistic was calculated.

Table 2.1: The t -statistic values and slope (ppm/minute) of regression between measured concentrations for: (a) CH₄, and (b) N₂O. H₀ was rejected for $t_{\alpha=0.025} \leq -3.184$ and $t_{\alpha=0.025} \geq +3.184$ for $n = 5$ (two-tailed test).

Set-up	(a) CH ₄				(b) N ₂ O			
	b	S_{YX}	$\sum_{i=1}^n (x_i - \bar{x})^2$	t_{value}	b	S_{YX}	$\sum_{i=1}^n (x_i - \bar{x})^2$	t_{value}
1	-0.056	8.673	228.804	-0.098	-0.0002	0.01000	0.000342	-0.00038
2	-0.111	4.686	78.109	-0.209	3.23E-05	0.00329	3.34E-05	5.69E-05
3	-0.059	4.849	73.997	-0.104	-6.8E-05	0.00235	2.11E-05	-0.00013
4	0.112	7.760	193.102	0.199	0.000301	0.00525	0.000174	0.000756
5	-0.008	2.877	24.881	-0.013	5.47E-05	0.00241	2.04E-05	0.000103
6	0.019	1.044	3.647	0.035	-1.3E-05	0.00438	5.77E-05	-2.3E-05
7	0.264	2.071	82.520	1.157	0.00026	0.00155	7.46E-05	0.00145
8	-0.005	3.115	29.133	-0.009	0.00037	0.00183	0.000149	0.00249

The test showed that the slope in the linear regression between concentration and time was not significantly different from zero, and that the set-ups could hold the gas for up to 40 minutes as desired for in these experiments. As a result, 40 minutes were chosen as the maximum time during which production samples were taken. None of the set-ups failed the hypothesis test, and thus from this set eight of the set-ups were selected randomly, and installed directly into the water bath that contained the tubs.

2.7 Conclusion

In this study, a system that could simulate soil temperature conditions under global warming conditions was successfully designed and tested, and could be adjusted to any desired temperature. The reliability and validity of the system was demonstrated over a period greater than twelve months without any breakdown. The system successfully replicated the desired soil temperature increases, but with very low diurnal variations. Such constant temperature in the whole soil profile may not be common in the real world of global warming. Nevertheless, compared to pilot attempts to develop a similar system based on heating pads, the set-up reported in this research enabled better control of soil temperature as desired, and was therefore used in the mechanistic studies of the effects of temperature on CH₄ and N₂O emissions from rice microcosms, as illustrated in the subsequent chapters. A typical system may also be used in the study of the feedback between global warming and greenhouse gas emissions from wetlands.

Chapter 3

Temperature Limits and Dynamics of Methane Fluxes from Rice Agriculture

3.0 Summary

Most studies have shown that soil temperature is positively correlated to CH₄ emissions, and this was confirmed using controlled greenhouse experiments in this research. The magnitude of the positive feedback is potentially large enough to affect the future atmospheric CH₄ concentrations. Above 28 °C, fluxes decreased with increasing temperature. This observation raises the question whether the Arrhenius model is right even below this temperature since the emissions are not from only biological processes. Nevertheless, the magnitude of the impact of projected global warming on CH₄ emissions from rice agriculture will vary regionally and latitudinally, given that the soil temperature for flooded rice varies from about 15 °C in northern latitudes to about 40 °C in equatorial wetlands. Because of the similarity of flooded rice paddies to natural wetlands, the overall projected change due to this feedback is likely to cause a significant change to atmospheric CH₄ concentrations in future.

3.1 Introduction

The current global CH₄ emission rate from natural and agricultural sources is about 40-50 % of the total CH₄ emissions (Whiting and Chanton 1993). Irrigated rice agriculture contributes an estimated 10-15 % of the total global CH₄ emission, and was probably contributed more in the past than now (Khalil and Shearer, 1993b). Among other factors,

temperature has been found to have a great effect on the rate of CH₄ emissions from the root zone to the atmosphere. Several studies have indicated that methane fluxes increase when the temperature was elevated (Holzapfel-Pschorn and Seiler, 1986; Parasher et al. 1993; Khalil et al., 1998c). The reported results vary from a marked influence of soil temperature on the methane flux with doubling of emission rates at a temperature increase of 20-25 °C (Holzapfel-Pschorn and Seiler, 1986) to diurnal change in fluxes correlated with temperature from field experiments (Khalil et al., 1998a,b,c; Schütz et al., 1989; Neue and Roger, 1994; Wang et al., 1997; Wang et al., 1999; Seiler et al., 1984; Schütz et al., 1990; Satpathy et al., 1997; Sass and Fisher, 1994). Under field temperature conditions, CH₄ fluxes increased with morning rising soil temperature, reached a maximum during early afternoon, and decreased rapidly during nighttime. The cause of this observation still remains a puzzle, and a subject of debate. While there is a general agreement in all studies that temperature plays a significant role, (I) the processes that are triggered by the change in temperature, resulting in the observed changes in CH₄ fluxes in heating experiments, and (II) the magnitude of this feedback, are not yet known.

The relationship between soil temperature and CH₄ fluxes has been quantified using the Arrhenius equation (Schütz et al., 1990; Wang et al., 1997, 1999:

$$F = A \exp\left(-\frac{E}{RT}\right),$$

Where F = flux, A = Arrhenius constant, E = Activation energy (in kJ/mole), and R is the universal gas constant (8.31 Jmol⁻¹K⁻¹).

The use of this model has also been extended to global climate models, with Q_{10} values between 1.5 and 2.5 being chosen for use in these models (Gedney et al., 2004). The relationship assumes that CH_4 emissions from rice agriculture will continue to increase exponentially with increasing soil temperature. While this assumption could be valid within the reported temperature ranges, various field and greenhouse experimental data do not support this assumption for temperatures above 30 °C. A close examination of results presented by Khalil et al. (1998a) and Parasher et al. (1993) indeed show that the tipping point lies between 28 and 35 °C (Fig. 3.1).

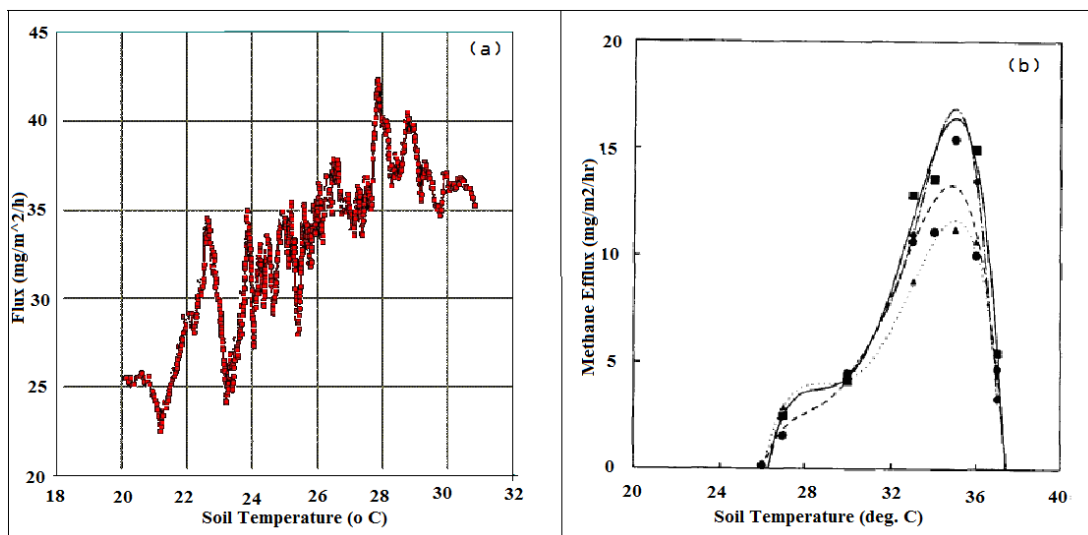


Fig. 3.1: (a) Rice field fluxes from Tu Zu China (reported in Khalil et al., 1998a), and (b) New Delhi, India (Parasher et al., 1993). Parasher et al. (1993) reported a distinct increase in CH_4 emission from rice plots with increase in soil temperature from 26 to 34.5 °C and a decrease in the rate of emission above the temperature under controlled soil temperature. Similar results can be observed in results reported by Khalil et al. (1991). All data indicate a sharp decline in CH_4 fluxes after reaching an optimum value or peak, an indication that the Arrhenius model will not consistently hold for temperature above 30 °C.

Similar results were observed in the previous greenhouse experiments (Sithole, 2009) and in plots of CH₄ flux versus temperature shown in Pangala et al. (2010). Because of the large variation in Q₁₀ values reported from various studies, the major challenge is the unification of these results. This limits the accuracy of model estimates of future global CH₄ emissions under a warmer world. To improve the quantification of this temperature feedback and model output, knowledge of the representative Q₁₀ value is required.

3.2 Aim and Objectives

In this experiment the aim was to design and run experiments that would be used to quantify the relationship between temperature and CH₄ fluxes and to determine how CH₄ fluxes will be affected by global warming.

3.3 Materials and Methods

Intensive experiments were conducted for two different rice growing seasons. To simulate climatic conditions under which the rice is grown, factors such as day length, day and night temperatures were controlled to match field environmental conditions in China. Since the planting density will determine the amount of fluxes, the plant density was maintained by multiplying the surface area of each tub to the field plant density.

3.3.1 Sample Collection

Gas samples were sequentially collected from Plexiglas flux chambers that were placed on top of planted and unplanted (control) tubs over 10-minute intervals (Fig. 3.1). This method has been used extensively in previous studies, and few problems associated with this method have been identified. To minimize these problems, (I) the length of time the chamber is placed on the rice, (II) the area and height of the chamber, (III) the frequency of measurements, and (IV) number of spatial replicates have to be considered (Khalil et al., 1998c). To avoid feedbacks and saturation effects on CH₄ concentration in the flux chambers (Khalil et al., 1998c), the exposure time was constantly maintained at 30 minutes, and the sampling frequency was about three to four days. It has been observed that a very low sampling frequency would increase the uncertainty of the seasonal flux (Khalil et al., 1998c; Khali and Butenhoff, 2008).

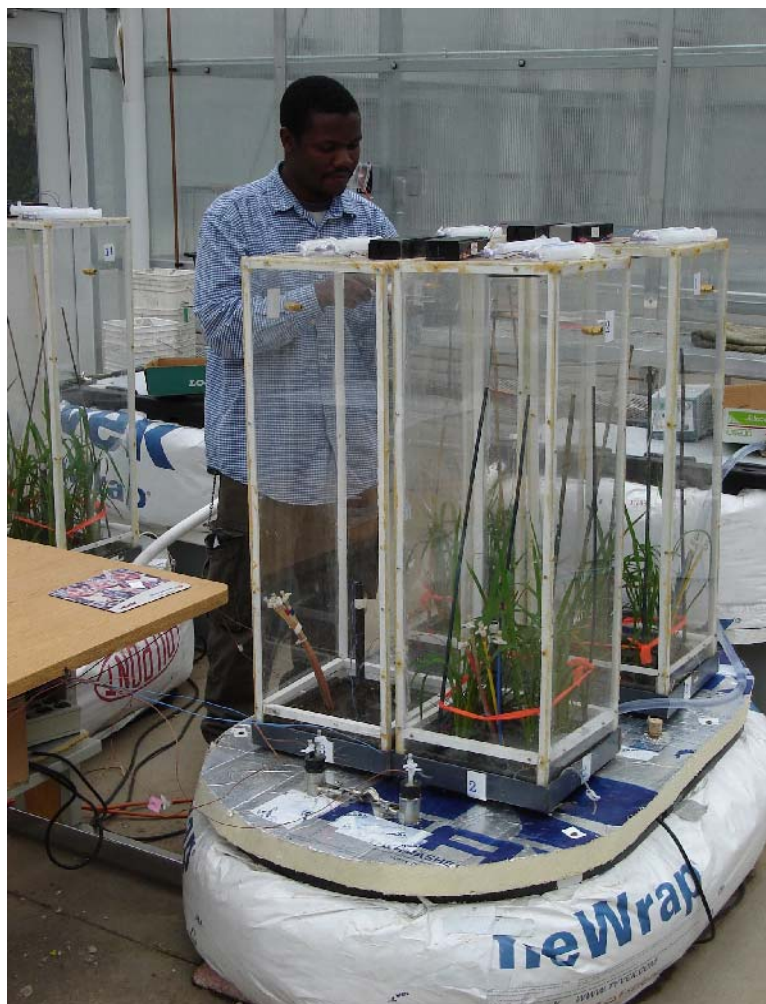


Fig. 3.2: Flux samples were collected from air-tight flux chambers in 10 minute intervals. Details of the construction of these chambers are presented in Chapter 2.

3.3.2 Data Analysis

The collected samples were analyzed with gas chromatograph (GC), equipped with a CH_4 flame ionization detector (FID) and an electron capture detector (ECD) as shown in Fig 3.3. About 5 mL of the gas sample was injected through the septum into four sampling columns of the GC, with nitrogen as the carrier gas. The net CH_4 fluxes F [in $\mu\text{g m}^{-2} \text{h}^{-1}$] were determined from linear regression of the observed concentrations with

sampling time. For the purpose of quality assurance, any slope not close to zero and the coefficient of determination ($r^2 \geq 0.90$) were checked for non-linearity (Khalil et al., 1998c).

The calculated net fluxes were corrected for tub headspace following the gradient technique (Khalil et al., 1998c):

$$F = \gamma \left[\frac{\rho M_w (V_{chamber} + V_{correction})}{A_{surface} N_o} \right] \frac{\Delta C}{\Delta t}$$

Where $V_{chamber}$, $V_{correction}$ and γ are volume of chamber, volume correction of tub headspace, and a factor equal to $6.0 \times 10^{-5} \text{ mg min}^{-1} \text{ gh}^{-1} \text{ ppbv}^{-1}$, respectively. $A_{surface}$ is the water surface area in the bucket.

Considering the flux, F to be a function of T and using the linear regression model:

$\ln(F) = \beta + \alpha T$ from which α was determined for the 90 % confidence limits. The daily Q_{10} was calculated as $Q_{10}(t) = e^{10 \alpha(t)}$. Where t is the time (DAT) when the sample was taken. The seasonal average Q_{10} was then calculated as:

$$Q_{10} = \frac{\int_{season} Q_{10}(t) \times F(t)}{\int_{season} F(t)}$$

Here F(t) is the base flux– that is the flux at the lowest temperature.

The uncertainty was calculated as $Q_{10}(\text{max}) - Q_{10}(\text{min})$ based on the 90 % confidence limits of the calculated Q10s for each day of the data.

3.4 Results and Discussion

3.4.1 Seasonal Flux Variations

Large temporal variations in CH₄ fluxes were observed. In planted tubs under different temperature treatments, CH₄ fluxes increased from zero at the start of the growing period, reaching maxima of between 20 and 60 mg/m²/hr during mid-season, and falling back to almost zero at the end of the season (Fig. 3.4). This work is consistent with the findings of Khalil et al. (2008c) in which values as high as 60 mg/m²/hr were observed under field conditions. The observed peak has been shown in previous studies, and has been attributed in part to the increase in root exudation, root and leaf surface area which would increase the efficiency of CH₄ transport from the soil to the atmosphere. The other factor is an increase in dissolved CH₄ pore water concentration.

This study also indicated that CH₄ fluxes were positively correlated with soil temperature at 10 cm depth, and that temperature was major driving factor in the increase of CH₄ fluxes, consistent with reports from other studies (Schütz et al., 1989). CH₄ fluxes differed greatly between different temperature treatments. Lowest CH₄ fluxes were observed at the lowest temperature set-up (20 °C), whilst the values were consistently high at higher temperatures throughout both seasons (Fig. 3.3).

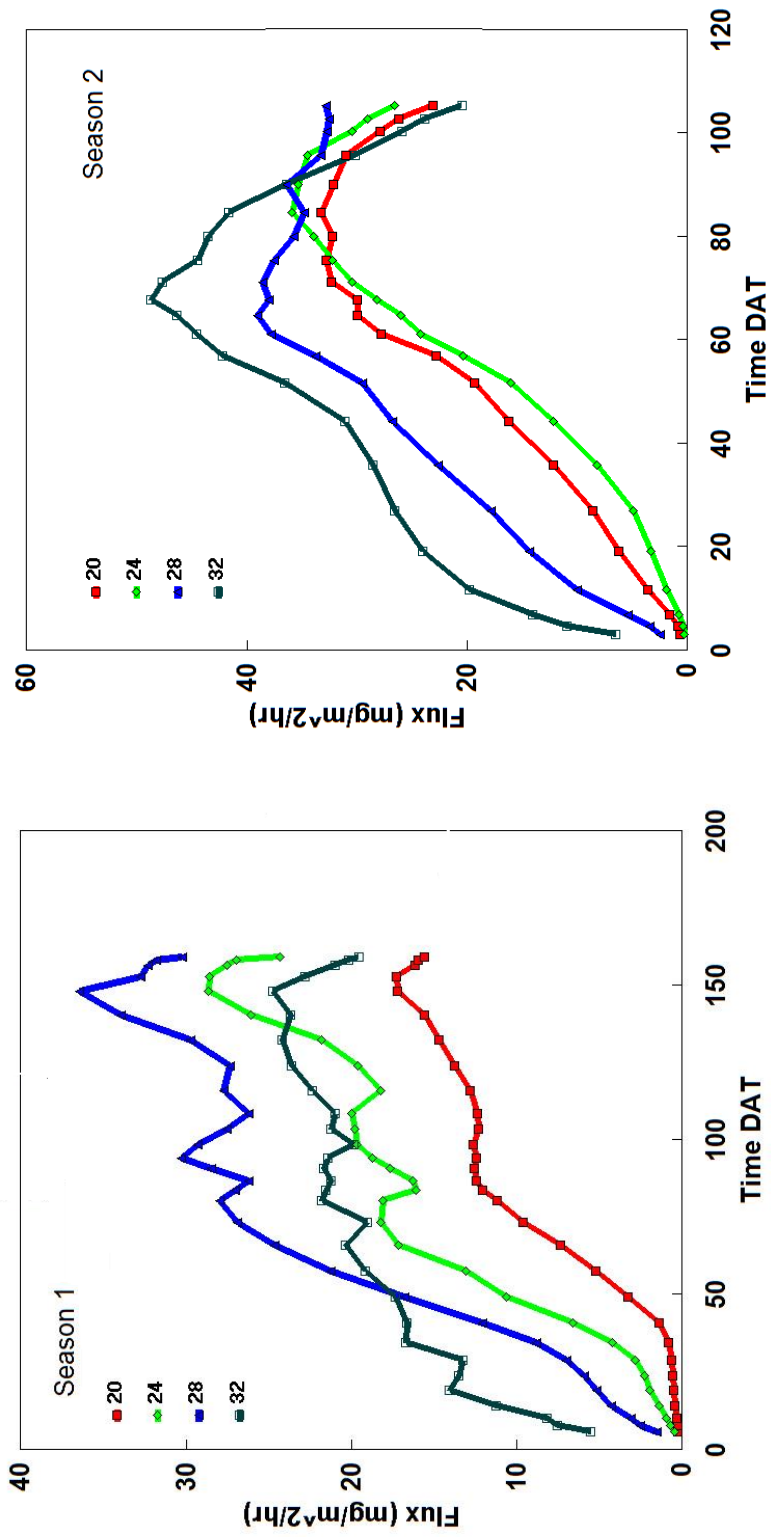


Fig. 3.3: Seasonal CH₄ fluxes across different temperature treatments and between winter and summer seasons.

This observation is not new; various studies have reported lower CH₄ fluxes to be associated with lower temperatures (Jiang et al., 2006). Several explanations have been given to account for the observed increase of flux with temperature. It is thought that the temperature around the roots greatly affects the CH₄ transport process in rice plants, and that the diffusion coefficient of dissolved CH₄ in pore water increases with increasing temperature. A factor of 1.5 times higher has been observed at 30 °C than that at 15 °C (Hosono and Nouchi, 1997).

In summer season, at 32 °C, CH₄ emission rates consistently increased at an accelerating rate at the beginning of the season, reaching a maximum during the mid-season, but falling below the emission rate at 28 °C. Similar results have been reported in other studies (Khalil et al., 1998; Parasher et al., 1993). CH₄ emission rates as well as soil temperatures showed a significant seasonal pattern. Between 10 and 50 DAT, CH₄ emissions increased by a factor of 2 for every 4 degree increase in soil temperature. Similarly, CH₄ emissions increases with successive increases in temperature between 5 and 20 °C were reported (Gauci et al., 2004) and between 20 and 28 °C. Such data has been found to fit the Arrhenius model from which apparent activation energies were calculated from correlations. The observation of this strong correlation has led to the general application of the model, irrespective of the temperature domain (Aselmann and Crutzen, 1990; Wang et al., 1997; Schutz et al., 1990).

However, these results show that for temperatures above 28 °C, fluxes declined. Early studies by Acharya (1935) reported by Neue and Roger (1994) found the optimum temperature to be 30-35 °C. Wang et al. (1997) found that raising soil temperature to about 30 °C sharply increased CH₄ emission rates, but not at 40 °C. They proposed that the low rate at 40 °C was because much of the CH₄ had already been emitted when the soil temperature was raised. However, these results and those presented in their paper, are all an indication of the breakdown between the Arrhenius projected (continuous flux increase with temperature increase) and experimental results. From these experiments, and analysis of results published in literature cited herein, it was observed that the Arrhenius equation generally fits best when the temperature is below the optimum, and above this point the relationship breaks down. First, it is important to note that these temperatures may not all be the same, at different soil temperatures. A correct model may be some other functional form that will be consistent with results of falling flux after a critical temperature.

3.4.2 Q₁₀ of CH₄ Fluxes

The Q₁₀ values were as high 34 at the start of the season and as low as 2 at the end of the growing season. The variation between temperature treatments was much higher during crop establishment (0-40 DAT) than the mid- to late season period (Fig. 3.5). The seasonally averaged Q₁₀s for Seasons 1 and 2 were found to be 2 and 1.7. These Q₁₀ values are similar to those found during the flowering stages, periods during which large fluxes were recorded.

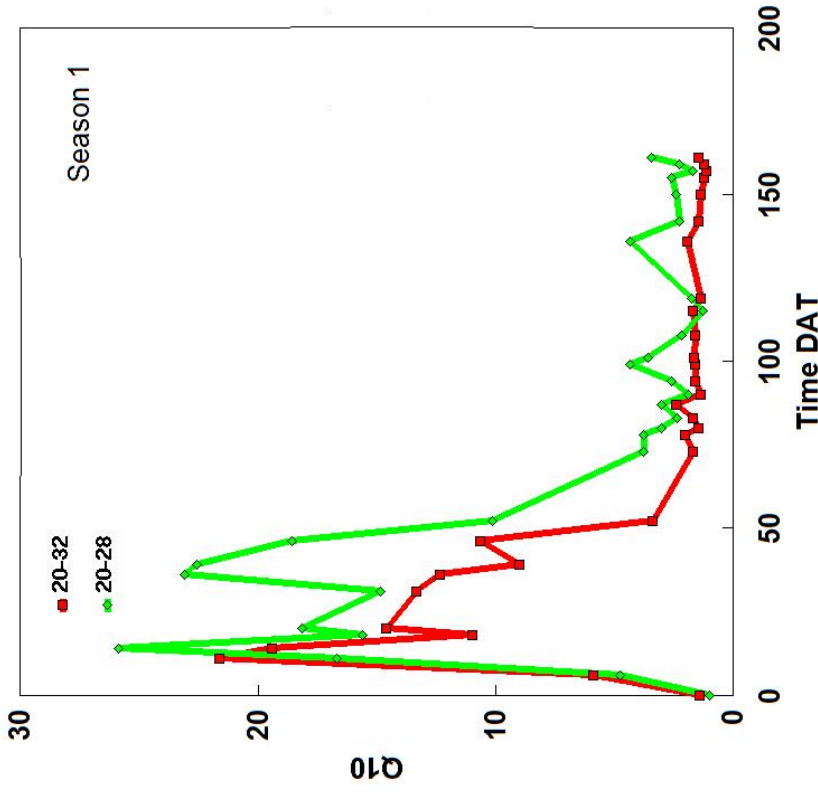
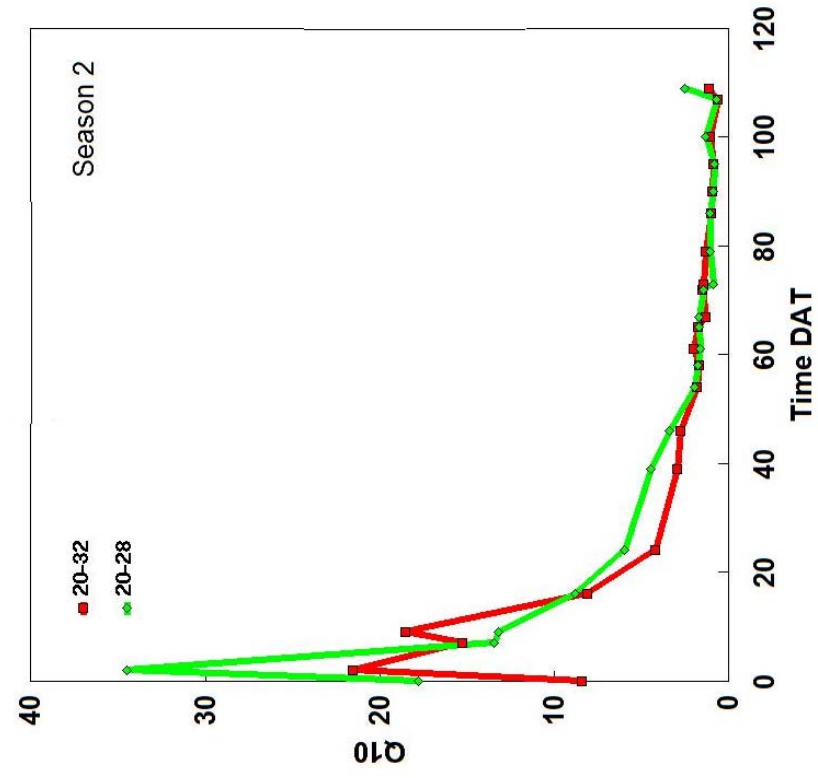


Fig. 3.4: Q_{10} of CH_4 fluxes for growing seasons.

While the Q_{10} may be high during the early period, the flux is very low so the overall effect of the high Q_{10} for the total seasonal emissions is not much. These seasonally averaged Q_{10} values are slightly lower than those in previous studies: 4.3, 3.1 and 2.9 (Khalil et al., 1998b), 4.9 (Yao and Chen, 1994b), and 3.5-4.0 (Sass et al., 1991). For example, Segers (1998) found an average Q_{10} of 4.1 from incubation experiments in which temperature was the single varying factor. Within these experiments, values as high as ~ 28 (Segers, 1998; Yao and Chen, 1994a), and 71 (Yao and Chen, 1994b) were recorded. Similarly, in three occasions, such values were recorded during the first three weeks after flooding and transplanting. An analysis of these Q_{10} values showed that very high uncertainties were common in the first three weeks after the start of the growing season (Fig. 3.5).

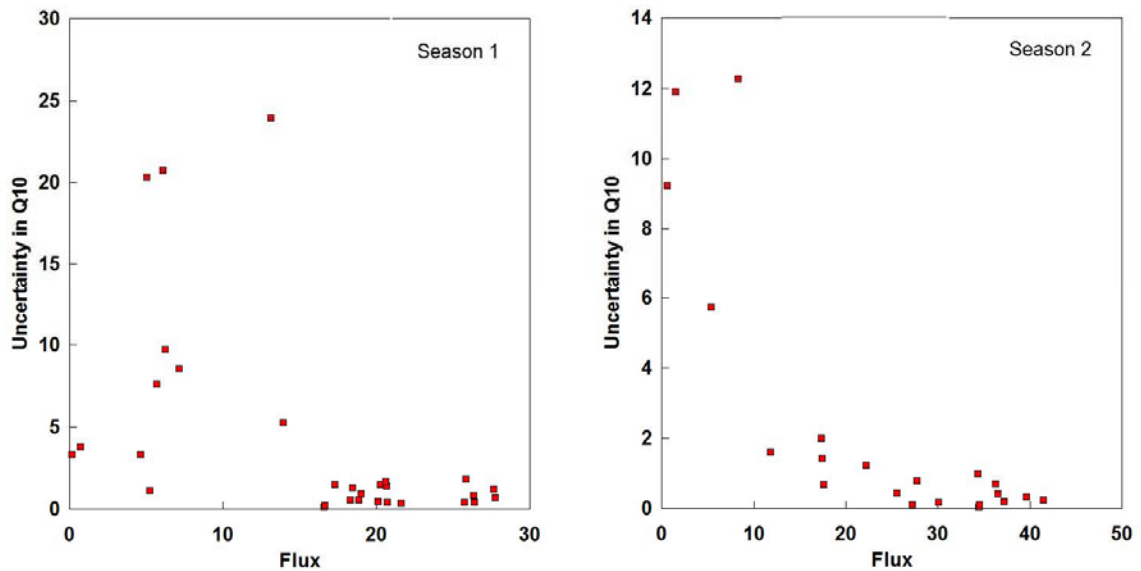


Fig. 3.5: Uncertainties in the Q_{10} of CH_4 fluxes during the growing season.

Segers (1998) proposed that the observed high Q_{10} values are a result of the effect of temperature increases on the rate of electron acceptor reduction, which lowers electron acceptor concentrations which have an additional positive effect on CH_4 production. However, it is noteworthy that during the first three week period, very low fluxes were measured (Fig. 3.6). Such highly variable values, including the low base fluxes, will result in large and highly variable calculated Q_{10} values. Hence these values are not a true reflection of the response between CH_4 fluxes from rice ecosystems. These high Q_{10} values could be representative of other processes such as ebullition, an artifact of experimental sampling procedures and calculations, or a composite of various unknown factors.

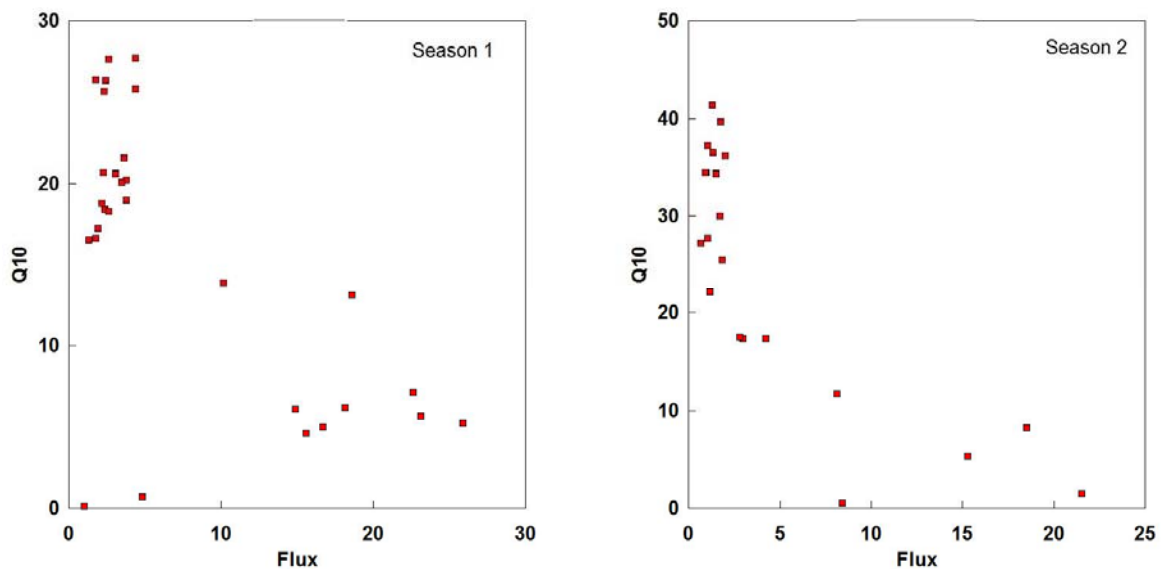


Fig. 3.6: Q_{10} of CH_4 fluxes during the growing season, showing high values of Q_{10} for low flux values.

Because of the large uncertainties, it is very difficult to insure that the observed change of flux is due only to temperature change. It is quite possible that some of the high Q_{10} s

occur because of other factors. Considering that the Q_{10} values calculated here are potentially a product of the Q_{10} s of methanogenic and methanotrophic activities, CH_4 production and oxidation (Khalil et al., 1998b), the Q_{10} values of these mechanistic processes will be determined and presented in the subsequent chapters.

3.5 Conclusion

CH_4 fluxes and Q_{10} values were found to be highly variable and dependent on the season. If these results are generally true for flooded rice ecosystems, the implication is that the net Q_{10} of CH_4 fluxes is base temperature-dependent (high for low base temperature and low for high base temperature) and changes with season. The other reason is that the Q_{10} varies by season because the underlying processes vary during the season. Considering that rice is grown in different regions and under a wide range of temperature regimes (15 °C in northern latitudes to 40 °C in equatorial region), the net Q_{10} of CH_4 fluxes cannot be represented by a single value when used in global upscaling.

Chapter 4

Dynamics of CH₄ Concentration in Pore Water under Elevated Soil Temperature

4.0 Summary

Concentrations of dissolved CH₄ in rice ecosystems were monitored to determine the effect of global warming on the concentration profiles and distributions to further elucidate the dynamics of how CH₄ is emitted to the atmosphere. The concentrations of CH₄ in pore water from four different temperature treatments at four different soil depths within the rice tubs were measured over two seasons using standard techniques. Our results suggested that CH₄ concentration is highly dependent on soil temperature, but only for a limited period during the rice growing season. In all the four temperature treatments, concentration values ranged from 1360 µg/L at the start of season to 7,290 µg/L at the end of the season, with highest and lowest mean concentrations of 3,373 µg/L (24°C) and 4,362 µg/L (at 28 °C), respectively. The highest concentration values were found between 10cm and 15cm. The concentrations at different depths were significantly correlated to fluxes from 11 to 60 DAT. During this period, the dependence of pore water CH₄ concentrations on temperature was more distinct than in any other part of the season. When CH₄ fluxes were compared to pore water concentrations for > 60 DAT, the fluxes flattened out around 30 mg/m²/hr despite increases in pore water concentrations. From this observation, we inferred that CH₄ transport capacity of the plant was the predominant limiting factor for fluxes during this plant growth phase.

4.1 Introduction

The CH₄ produced from anaerobic decomposition of soil organic matter (Conrad 1989; Conrad, 1993; Rothfuss and Conrad, 1993) is oxidized in the floodwater and some is emitted to the atmosphere, while the remainder is trapped in soil-water solution as dissolved methane (Alberto et al., 2000). Dissolved CH₄ is the primary source of CH₄ emissions from rice agriculture into the atmosphere (Aselman and Crutzen, 1989; Rothfuss and Conrad, 1993; Alberto et al., 2000). Dissolved CH₄ in pore water is an important intermediate of CH₄ emissions in rice paddies. The longer the residence time of CH₄ in the soil, the more likely they will be oxidized by methane-oxidizing bacteria (Alberto et al., 2000) and thus, reduce CH₄ emission to the atmosphere.

Despite countless measurements of CH₄ emissions over the past four decades, few studies have focused on the effect of temperature on CH₄ pore water concentrations. In the majority of the studies, less than 10 % of the reported results have been devoted to CH₄ pore water concentration, and hence the likely effect of global temperature increase is poorly understood at present.

In rice paddies, CH₄ release into the atmosphere depends on the production, consumption and transport from anoxic zones (Conrad 1989; Wilson et al., 1989; Schultz et al. 1989; Conrad 1993; Alberto et al., 2000; Schultz et al., 1989; Sass et al., 1990; Yagi and Minami 1991; Khalil et al., 1991). Differences in pore water CH₄ concentrations have been reported from field experiments performed during winter and summer seasons (Borken et al., 1999). In a similar way, other studies have shown diel variations vertical

profiles, with increased CH₄ concentrations following sunrise, reaching maxima around 9:00 AM within the 5–15 and 25 cm depths, and at 12:00 PM for 30–35 cm depths (Ding et al., 2004). The results indicated that temperature is an important factor for CH₄ concentration in pore water, among other environmental factor. However, due to heterogeneity of various factors between the seasons, it is important to perform the experiments under carefully controlled conditions in order to understand these factors, and hence develop the means towards reducing CH₄ emissions from rice paddies.

Increases in soil temperatures due to global warming may cause significant changes to the rates of fundamental processes that produce CH₄ in rice paddies. To understand these impacts, it is important to make an assessment of the CH₄ pool in rice paddies.

4.2 Aim and Objectives

The present experimental study was designed to: (I) quantify the effect of temperature, (II) determine the seasonal characteristics of dissolved CH₄ in rice paddies, and (III) to evaluate CH₄ concentrations in rice paddy soil. The objective was to understand how an increase in temperature would affect the CH₄ pool from which CH₄ is released into the atmosphere.

4.3 Materials and Methods

4.3.1 Introduction

Pore water CH_4 concentrations were determined by sampling pore water at four different depths during the winter and summer seasons of 2010. The samples were collected through plastic sampling tubes that were installed in the soil-filled tubs as shown in Fig.

4.1.

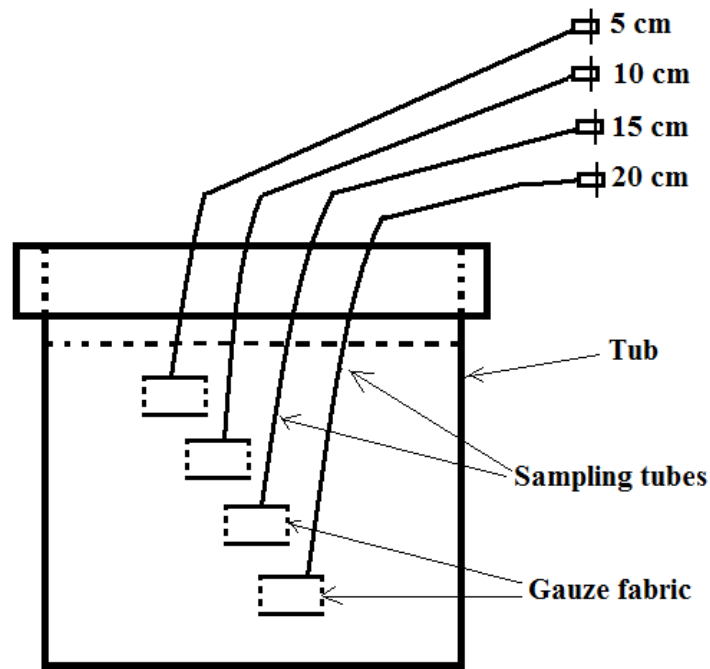


Fig. 4.1: Schematic diagram of pore water samplers (Khalil et al., 2008) inside the tubs. The samplers were inserted at 5, 10, 15 and 20 cm soil depths in each of the tubs. The cylinders were covered with gauze fabric to allow water movement between their interior and the surroundings.

Rice seedlings were immediately transplanted after fertilization and flooding. Samples were taken three days following transplanting rice and every week thereafter, and analyzed for dissolved CH_4 .

4.3.2 Sampling Method

Before taking samples, about 10 mL of pore water was flushed out of each sampler to remove trapped air and impurities in the plastic tube lining. After some 30 minutes, 15 mL of pore water was drawn-out from each tube using the syringe. Every time a sample was drawn, the valve on the tubing was closed immediately after disconnecting the syringe from the tube.



Fig. 4.2: Pictures of pore water tubes embedded into flooded soils inside tubs containing temperature probes. The tubes were fitted with valves to which the syringes were hooked during sampling.

Trapped air bubbles inside the syringe were immediately removed by slightly tapping and flushing out some of the pore water with the syringe nozzle facing upwards leaving

exactly 10 mL of the sample pore water. Maintaining the syringe in that position, the valve was closed to prevent any contamination due to exposure to ambient air. Immediately after collection, the 10 mL samples were taken to the laboratory and the temperature of the 10 mL samples were brought to room temperature.

4.3.3 Sample Analysis and Measurements

Samples were returned to the laboratory and analyzed the following day. For analysis, to each 10 ml sample, 20 mL of 99.9 % N₂ was added (Khalil et al., 1998). The gas-solution-mixture was shaken vigorously for 5 min to drive dissolved CH₄ from pore water into the headspace (Ding et al., 2004).

The headspace gas was collected and analyzed for CH₄ using an FID and ECD equipped GC. Concentrations of the dissolved CH₄ in headspace were determined as outlined in Lu et al. (2000):

$$[X]_{dissolved} = \left[\frac{[X]_h \times (V_h + PV_s) - [X]_a V_h}{16V_s} \right] \times \rho_x$$

Where $[X]_h$ and $[X]_a$ are the headspace and the ambient air concentrations (in μmolL^{-1}); V_h and V_s are the headspace volume and soil solution volume (mL), respectively. P is the partition coefficient (0.03 mL air mL⁻¹ water at laboratory temperature), ρ_x is the density of the gas, respectively.

4.4 Results and Discussion

4.4.1 Profiles of dissolved CH₄ in the rhizosphere

Results indicated that from 0 to 15 cm, there was a general increase in the concentration of dissolved CH₄, with slight differences between different temperature treatments (Fig. 4.3). Similarly, Rothfuss and Conrad (1993) reported CH₄ concentrations that increased with depth, reaching a maximum in 5 – 13 cm soil layer. Studies under both field and greenhouse conditions have shown similar vertical distributions in CH₄ concentrations (Gross et al., 1993; Sebacher et al., 1985; Liblik et al., 1997) and profiles of methanogenesis in the submerged paddy soil (Schültz et al., 1989). In all four treatments in this study, highest concentration values occurred between 5 and 15 cm, except in the 24 and 28°C where the highest values occurred at 20 cm. Using a photoacoustic technique, the same results were observed in a paddy field (Rothfuss et al., 1996). In other studies on an Italian rice field, from vertical profiles of the respiratory index, Rothfuss and Conrad (1993) found that acetate was more degraded by methanogenesis in 5 - 11 cm soil depth compared to other soil layers, an indication that this layer is generally the zone for CH₄ production (Lu et al., 2000).

Comparing different temperature treatments, highest concentration values occurred in the 32 °C tubs. The profiles showed that over the whole season, the average dissolved CH₄ in the pore water was generally higher in the 10-20 cm zone than in the 5-10 cm layer, consistent with what has been reported in other studies (Wilson et al., 1989; Schultz et al., 1989). It is important to note that the 5-10 cm zone contains more oxygen and higher populations of CH₄ oxidizing-bacteria than other layers. Thus, as the season progressed,

the number of CH₄ oxidizing bacteria would also respond, depending on the supply of methanogenic substrates (Schutz et al., 1990) and temperature. In the 32 °C temperature set-up, CH₄ concentrations were much higher in the 10-15cm layer. This dependence on temperature could also be due to stimulation of bacterial population growth (Holzapfel-Pschorn et al., 1985; Dunfield et al., 1993) or enzyme synthesis that results in the production of CH₄ (Schutz et al., 1990), or a combination of both. High seasonal fluctuations were also common in the near-surface zone (0-5 cm).

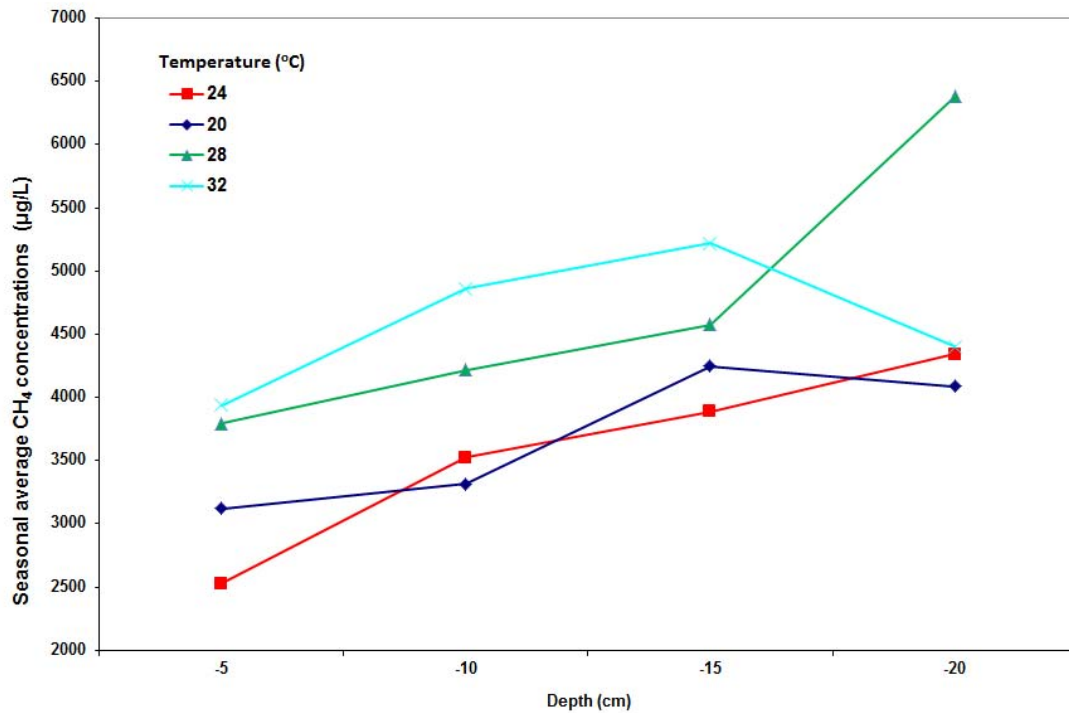


Fig. 4.3: Temperature effect on the seasonal average CH₄ concentration vertical profiles in the soil water in for planted tubs. In all four different temperature treatments, the average values are close to each other, with highest values at 20 cm depth.

The variations could be due to changes in surface water temperatures and supply of organic materials (Wilson et al., 1989). Typically, such fluctuations have been attributed

to the high variations in the supply of organic materials (such as leaf litter) than in the levels below.

The high concentrations at the bottom could also be due to transport processes. At the bottom there is no flux, while all the flux is at the top. So even if there is no production at the bottom the concentration would be high. Throughout the seasons, the average dissolved CH₄ concentrations in all treatments were lowest at 5 cm depth, followed by that at the 20 cm depth, except in one particular case in which the highest occurred at 20 cm (Fig. 4.3). However, when only the 0-60 DAT was considered, the average CH₄ concentrations of the 32 °C treatment at 10-15 cm depth were about three times higher than that at 20 °C (Fig. 4.4). This indicates that the rate of accumulation of CH₄ was highly temperature dependent during the start of the season. In one study in which light treatments were used, the same effect was observed, with vertical profiles showing higher concentration in light- than dark-incubated treatment (Rothfuss et al., 1996). The observation was attributed to differences in the response of photosynthetic rates of algae to light. However, temperature measurements in these treatments were not reported or inferred. From these results (planted and un-planted tubs), it was inferred that temperature is the major factor that influenced the observed rate of increase of CH₄ in pore water. On the other hand, the concentration within this zone was about 1.5-2.0 times higher than in the other two zones, indicating that production in flooded soil generally occurred between 10 and 15 cm depth. As the season progressed, the parabolic profile got distorted, as the profile shifted towards the linear case, in which the highest concentration occurred at 20 cm and the lowest at 5 cm. This shift can also be noted in field pore water

CH₄ concentration profiles reported by Rothfuss and Conrad (1993). This transition could be due to CH₄ accumulation under limited transport and oxidation processes.

In all treatments, differences of CH₄ pore water concentration profiles were also apparent between planted and unplanted tubs during the period 0-30 DAT, and diminished thereafter. Similar shifts were noted for CH₄ concentration profile results between DAT 21 and 40 reported in Landu (1994). At >60 DAT, highest concentrations occurred in unplanted treatments similar to observations in Gross et al. (1993).

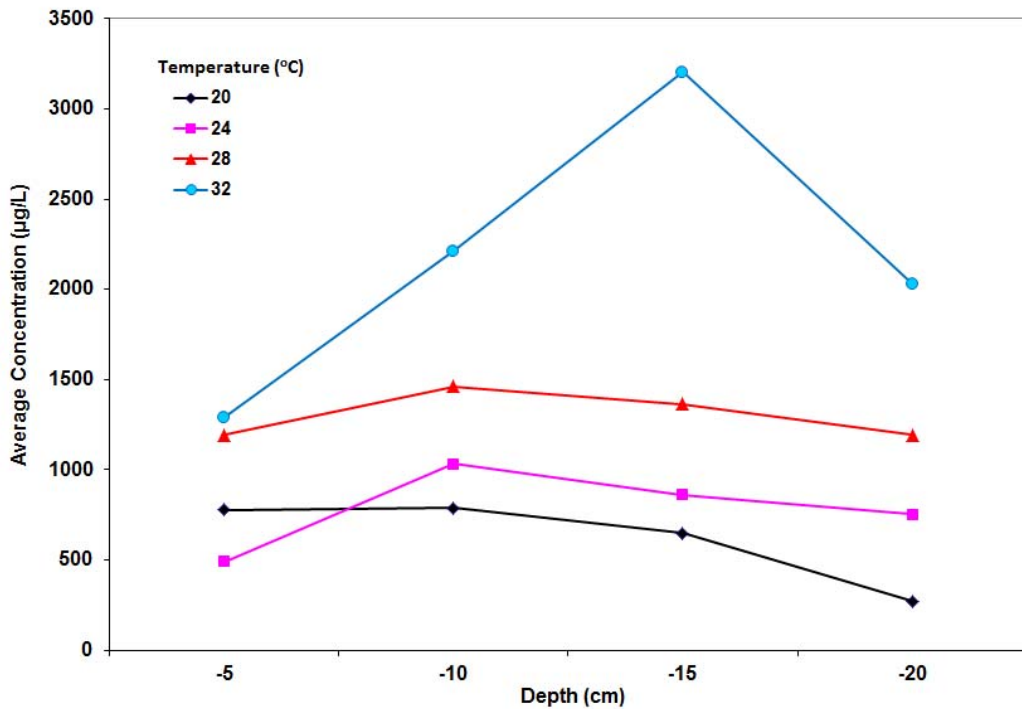


Fig. 4.4: Vertical pore water CH₄ concentrations profiles for 0-60 DAT, indicating layering in different temperature treatments. In each treatment, highest values occur between 10 and 15 cm. Differences in the profiles shows that temperature is an important factor during this phase of the season.

These higher values in planted tubs during this part of season could be due to additional CH₄ produced from roots exudates (Schutz et al., 1990), root/rhizome distributions (Gross et al., 1993), while the low values after mid-season could be due to the effect of transport mechanisms from the soil to the atmosphere and O₂ transport into the root zone through plants (Schutz et al., 1990; Lindau, 1994; Lu et al., 1999; Lu et al., 2000).

In bare tubs (not shown), no distinct parabolic profiles were observed. Seasonally, the concentrations of CH₄ in the unplanted tubs were more variable over time than those in planted tubs, particularly in the 5 cm zone. However, just as in planted tubs, the minimum values were consistently found with the 5 cm zone, with highest values at 20 cm. It was also found that the pore water CH₄ concentrations in the planted tubs were slightly lower than those in control tubs, except within the production zone. It was inferred that the production of CH₄ and its accumulation was more governed by availability and population size of both CH₄ producing bacteria and readily decomposable organic material (Davidson and Janssens, 2006; Bridgham et al., 1995; Inubushi et al., 2002; Khalil et al. 1998; Dunfield et al., 1993; Sass et al., 1991), root exudates, and changes in the effectiveness of plant roots as conduits for both O₂ into the soil and CH₄ into the atmosphere (Gross et al., 1993).

4.4.2 Temporal Variations

Results showed that in all treatments, dissolved CH₄ concentrations were lowest at the time of transplanting and increased as the season progressed. The low values at the start of the growing season have been attributed to elevated soil redox potential and

adjustment of microorganisms to flooded soil conditions (Lindau, 1994). During the first two months after transplanting, pore water CH₄ concentrations were highly dependent on temperature - the higher the temperature, the higher the concentration during the 0-60 DAT. The differences in the seasonal temperature response of rate of accumulation of CH₄ in pore water could be due to the increases in methanogen populations (and their metabolic rates), methanogenic substrates (Wilson et al., 1989; Lu et al., 2000), and self-limiting processes such as transport and oxidation. However, over the season, there was no significant difference between CH₄ concentrations in the pore water across the four temperature treatments. Temporally, the concentrations at different layers showed very distinct weekly variations. Typical variability has been reported in various studies (Gross et al., 1993; Liblik et al., 1997). CH₄ concentrations generally increased from the onset to mid-season stage, and thereafter, the concentrations decreased gradually till the season ended. Similar trends were observed at all layers and different temperature set-ups. The decline in CH₄ concentrations in both planted and unplanted tubs towards the end of the season has been attributed to the decrease in the pool of decomposable dissolved organic material (Kimura et al., 1993; Lu et al., 2000) and population of CH₄ consuming bacteria in the soil (Dunfield et al., 1993). Despite the variations in the mean seasonal concentrations at different layers, the trends were similar for 10-20 cm soil layers and temperatures. However, there were variations across different temperature treatments in the 0-5 cm soil horizons.

Seasonally, the differences in the mean concentrations were statistically the same between concentrations in 20 and 24 °C temperature zones. Significant differences were

however, noted between low temperature treatments and concentrations in the 32 °C tubs. These observations suggest that the optimum temperature for production is somewhere close to this temperature.

Contrary to studies by other researches, there was a significant relationship between CH₄ concentration and soil temperature at all depths during the first half of the season, with stratification between different layers. In general, the correlations between CH₄ concentration and soil temperature at different depths were weak. Seasonal patterns of CH₄ fluxes closely followed the same dynamics of CH₄ concentrations between 0 and 60 DAT.

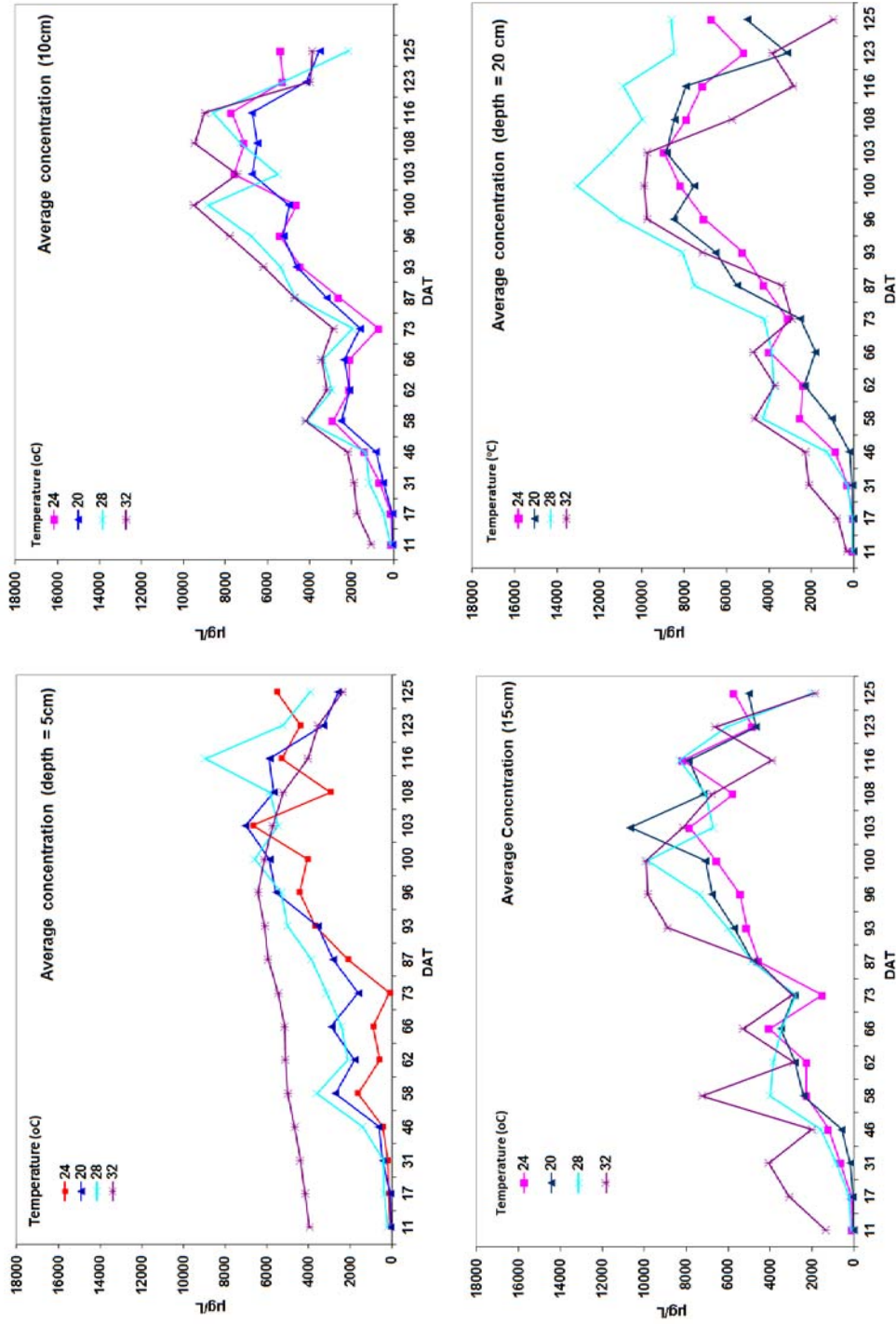


Fig. 4.5: Effect of temperature on CH₄ concentration in pore water, showing higher concentrations at 32 °C at the beginning and lower at the end of the growing season. Lowest concentrations were present at lowest temperatures. Concentrations increase at the start of the growing season, reaching a peak at mid-season, and decrease as the season ended.

In control (bare) tubs, similar trends, but higher concentrations (~11,000 $\mu\text{g/L}$) were observed than in rice planted tubs (Fig. 4.5). In a similar fashion, the effect of temperature was apparent. The 32 °C tubs had the highest CH_4 concentrations, while the 20 °C had the lowest values in the 0-73 DAT period. Beyond this period, there was no discernable indication of temperature dependence.

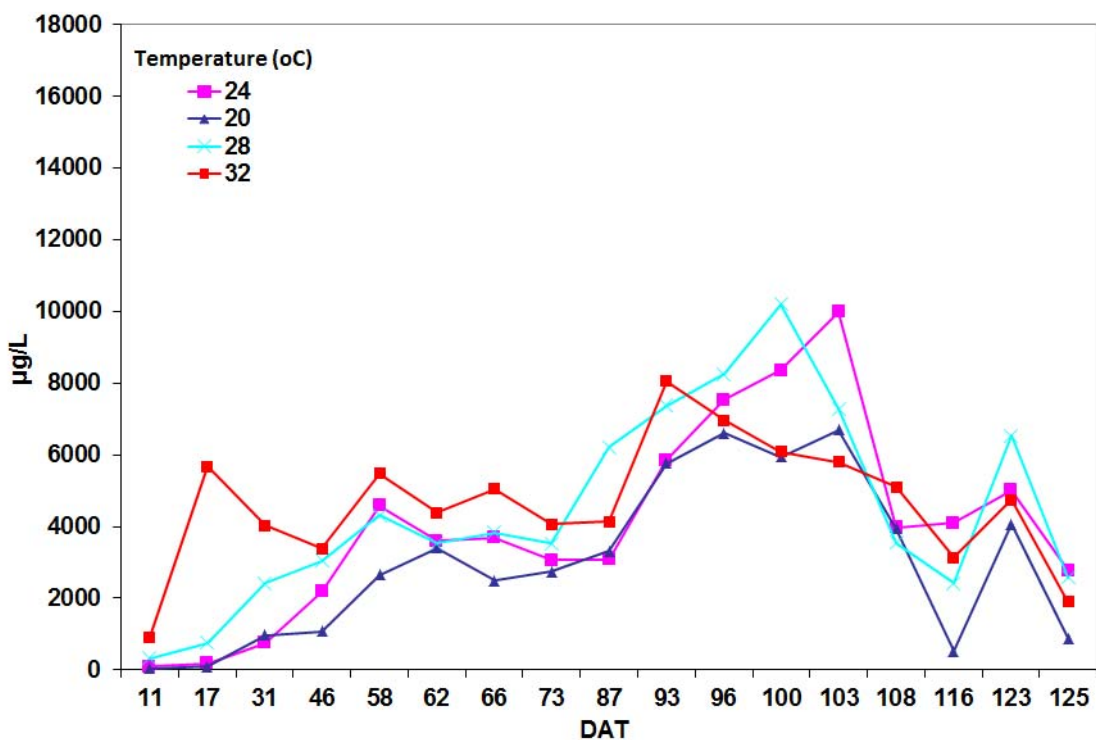


Fig. 4.6: Average concentration of CH_4 is higher at 32 °C at the start and lower at the end than at other temperatures. Concentrations were low at 20 °C throughout the season.

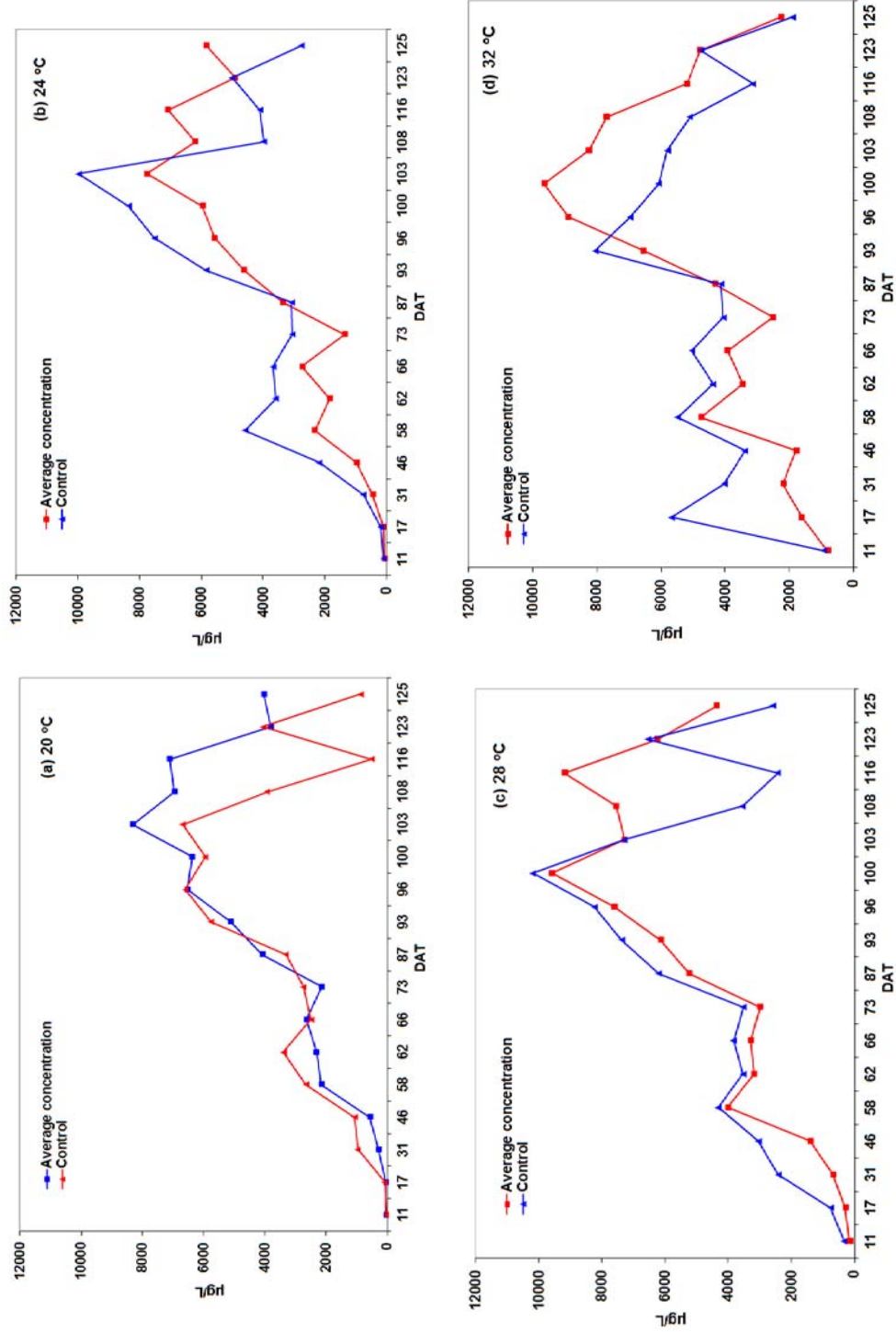


Fig. 4.7: Average concentration per temperature set-up. Effect of transport and temperature on pore water CH₄ concentrations. Generally higher concentrations of CH₄ in control tubs at the beginning of the season, but lower at the end. These observations could be due to bacteria degrading decaying roots.

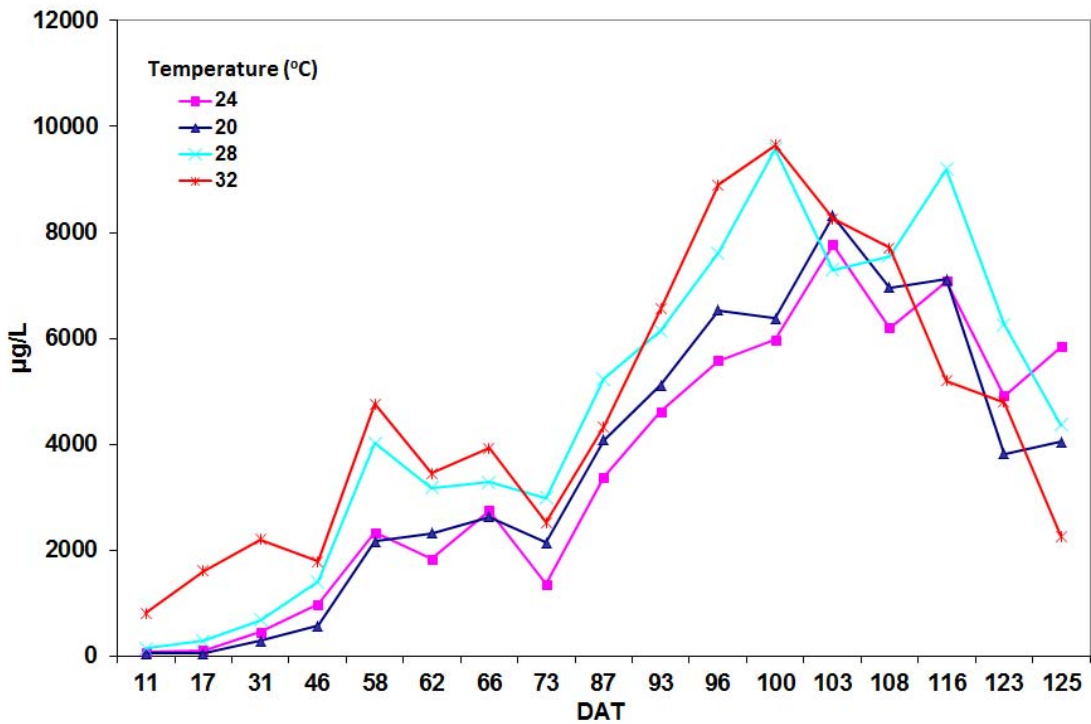


Fig. 4.8: Trends of pore water CH₄ concentrations across different temperatures. Concentration of CH₄ at 32 °C is higher till mid-season, but lower than that at 28 °C thereafter. This is consistent with flux observations, in which fluxes have been found to be higher at for 32 °C at the start of season, but quickly diminished after mid-season, with concentration at 28 °C becoming higher beyond this point.

Consistently, higher pore water CH₄ concentrations were associated with higher temperatures (Fig. 4.7). This was an indication that the temperature effect on the relationship between pore water CH₄ concentration and fluxes seem to exist between 0 and 60 DAT. In one observation (5cm, 32 °C), average pore water CH₄ concentration was much higher in all than other in other treatments (Fig. 4.7, Fig. 4.8).

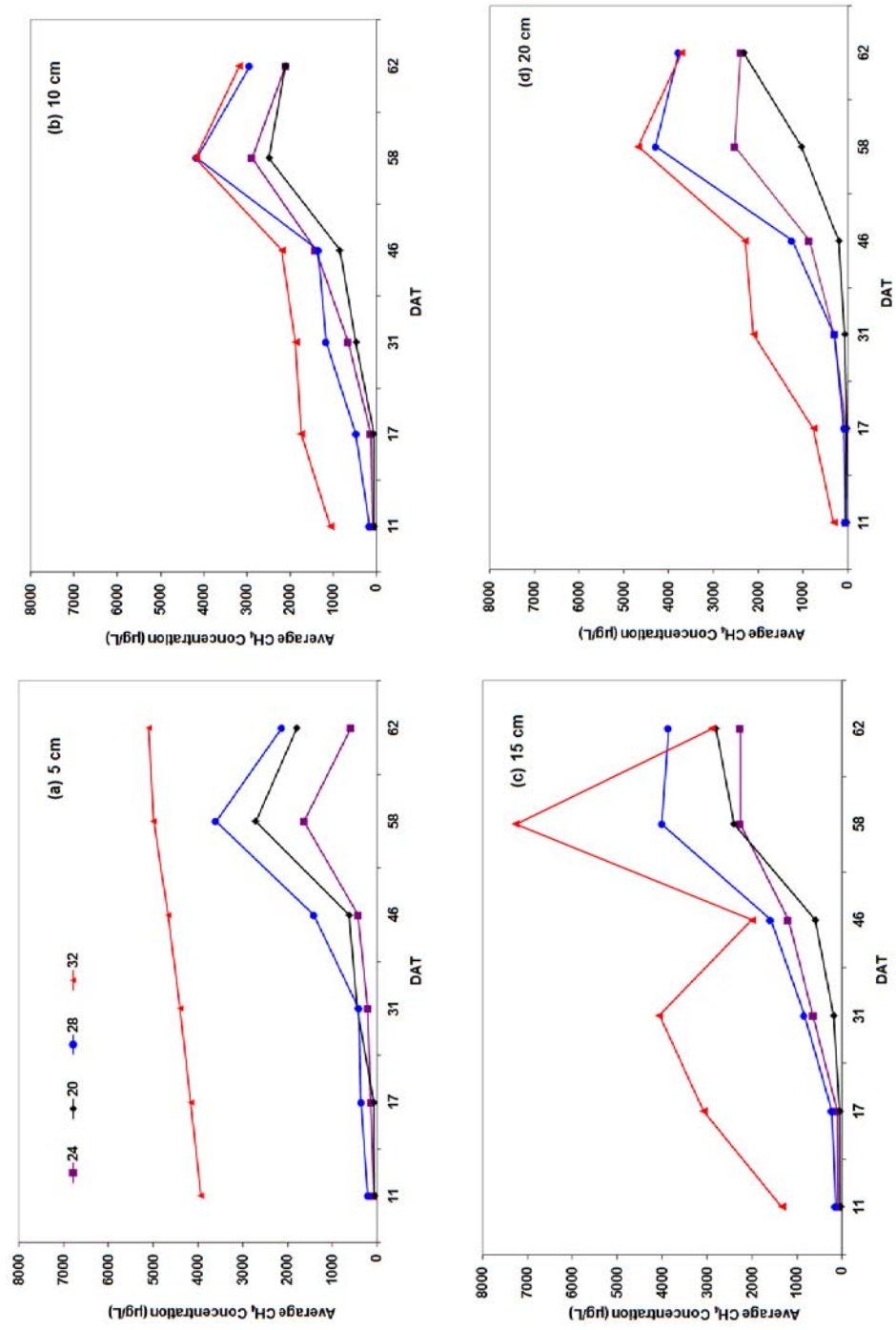


Fig. 4.9: Between 1 and DAT 60, pore water CH₄ concentration was the limiting factor in CH₄ fluxes. During this period fluxes increased to a maximum. In all treatments, CH₄ concentrations were highest and lowest at 32 °C and 20 °C.

Emission rates from rice fields are correlated to CH₄ concentration in the pore water during the first half of the season. Between 1 and DAT 60, pore water CH₄ concentration (Fig. 4.9) was the limiting factor in CH₄ fluxes (Fig. 4.10). This limiting point could be an indication that fluxes remain almost constant even though CH₄ pool increased in the pore water.

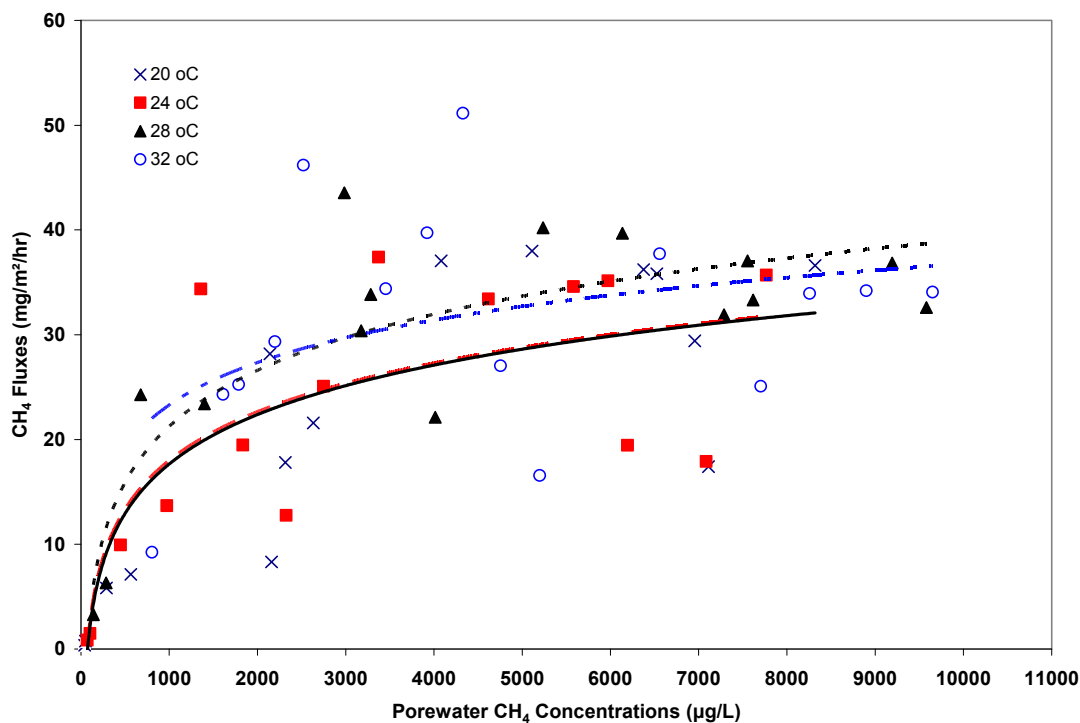


Fig. 4.11: CH₄ fluxes reaches maximum (25-40 mg/m²/hr) at 60 DAT (while the pore water concentrations increased from 60 µg/L to 1000 µg/L), and then level off despite continuous increase in CH₄ pore water concentration.

However, the rate of growth was almost constant till mid-season, an indication that increasing temperatures beyond the 28 °C would not result in continuous increase in the

rates of pore water CH₄ concentration accumulation. This implies that although the process that produces CH₄ is temperature-dependent, this process is limited by other factors. One possible factor may be that as the growing season progresses, the O₂ from the roots oxidizes the Fe(III) around the root system, resulting in the formation and accumulation of Fe(III) oxide precipitates around the roots thereby reducing the root surface area over which the CH₄ can diffuse into the root system.

4.5 Conclusion

Pore water CH₄ concentrations were successfully monitored at different soil temperatures. It was noted that temperature is a limiting and an important factor during the first two months after transplanting. During this period, temperature affected the rate of growth of both fluxes and pore water concentrations. As a result, fluxes also increased with increased dissolved CH₄ in the floodwater. The results also suggest that once the CH₄ pool in the root zone reaches a value of about 1000 µg/L, fluxes will depend on the capacity of the rice plants to transport CH₄ to the atmosphere. On the other hand, these results also indicate that the CH₄ transport rate in rice plants is a self-limiting process, even though it depends on the plant and root development and temperature among other environmental factors.

Chapter 5

Temperature Dependence of Methane Production

5.0 Summary

The effect of temperature on CH₄ production in rice paddies was investigated using standard incubation techniques. Experiments were performed on four different soil water bath-controlled temperature treatments (20, 24, 28, and 32 °C) under continuously flooded conditions. Soil temperatures were continuously measured during the growing season using thermocouples and microloggers. Results showed that after two weeks following flooding, the rate of CH₄ production was highly dependent on soil temperature over the season. The calculated seasonal Q₁₀ of CH₄ production varied from 2.1 (lower limit) to 3.3 (upper limit), with an average value of 2.6 (90 % confidence level). No seasonal dependence of Q₁₀ value was observed in all treatments. If this observed effect of temperature is representative of all rice paddies, and possibly wetlands, then global warming is likely to enhance the production of CH₄, and consequently emissions if oxidation does not increase by the same factor. Considering that the processes involved in the production of CH₄ in rice ecosystems is similar to that in natural wetlands, the atmospheric CH₄ contribution due to global warming feedback is likely to change the current total biotic CH₄ contribution.

5.1 Introduction

The most fundamental process in the emission of CH₄ from rice agriculture is the production process. However, the rate of CH₄ emissions from these ecosystems is a

composite of production, transport and oxidation processes. In rice paddies, CH₄ is produced as a terminal step of the anaerobic breakdown of organic matter (Schütz et al., 1989) by a group of microorganisms called Archaea (Yao and Conrad, 1999; Mitra et al., 2002). This process is driven by a complex food chain of various anaerobic bacteria de-polymerizing and fermenting organic matter (Schütz et al., 1989), resulting in the production of volatile acids and eventually CH₄. These methanogenic bacteria utilize substrates such as H₂/CO₂, formate, methanol, methylamines, acetate for growth and CH₄ production (Conrad 1989; Schütz et al., 1989) under free oxygen and at redox potentials of less than -150 mV and optimal pH of 6-8. Thus methanogenesis depends on substrate availability such as amounts of easily degradable organic matter, reducible Fe(III) and sulfate (Watanabe, 1984), and many other environmental factors such as temperature (Tsutsuki and Ponnampereuma 1987). The most important factors that control CH₄ production are soil type, rice variety, temperature, soil redox potential, water management and organic carbon or nitrogen (Conrad, 2002). This organic matter is derived from root exudation, sloughed-off root cells and decay of roots (Conrad, 2002). The production and oxidation of CH₄ in flooded rice soils are controlled by many soil, plant, and microbial factors. CH₄ production rates of soils are significantly and linearly correlated with soil organic matter content (Wang et al., 1999). The extent and duration for which CH₄ production is suppressed mainly depends on the ratio of available organic matter to reducible ferric iron (Yao et al., 1999; Watanabe and Kimura, 1999). Rice plants affect CH₄ in the root zone by providing methanogenic substrates through root exudates, decaying root tissues, and by respectively transporting O₂ and CH₄ into and out of the root zone through their aerenchyma.

Methanogens produce CH_4 from either acetate or H_2/CO_2 (Conrad, 1989, 1999). Acetate, H_2 , and CO_2 are byproducts of the microbial degradation of organic matter under anoxic conditions. Acetate can either be derived from root exudation or from fermentation (Aulakh et al., 2001).

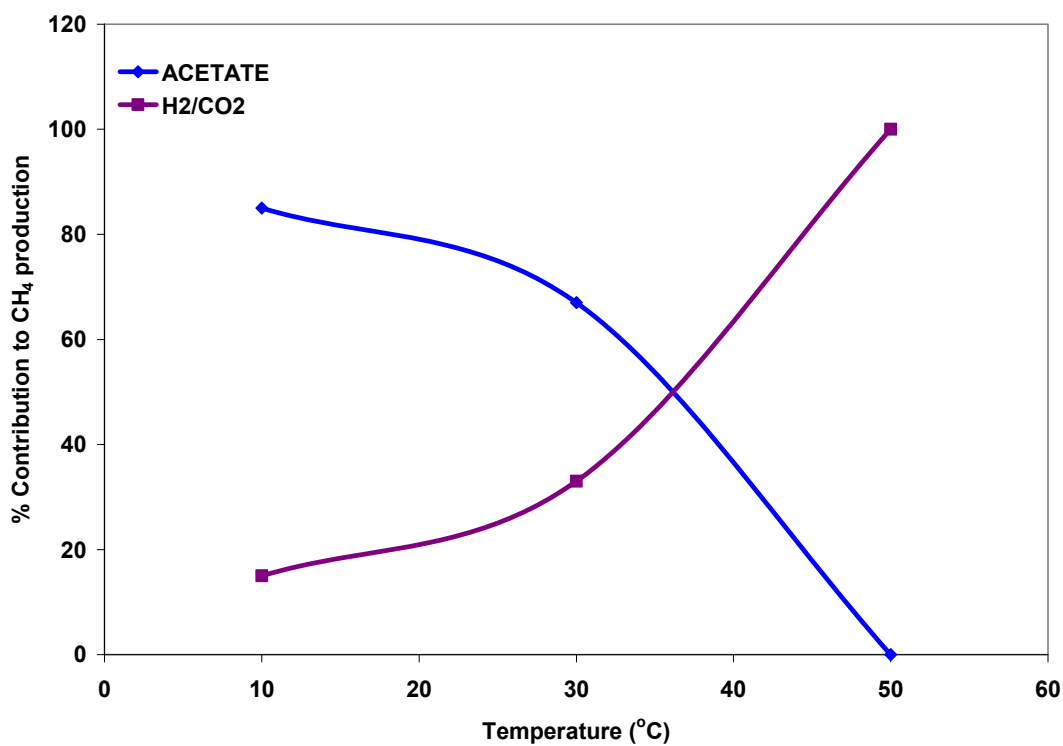


Fig. 5.1: Effect of temperature on the percentage contribution of acetate and H_2/CO_2 to methanogenesis in anoxic rice soil. The data shows that as temperature was continuously increased above 35 °C, CH_4 production contribution from H_2/CO_2 became predominant, while that from acetate diminished. Data extracted from Conrad (2002).

CH_4 production is also inhibited by NO_2^- , NO and N_2O that accumulate during the reduction of nitrate produced from applied chemical fertilizers. Yao et al. (1999) also found that maximum CH_4 production rate was related to both the soil oxidizing and

reducing capacities. CH₄ production is also affected by certain microbial populations that produce methanogenic substrates (Conrad, 2002). Exposure to O₂ also inhibits the population growth of methanogens, and hence limits CH₄ production. Among environmental factors, temperature has been identified to be very important to the production of CH₄ in flooded soils. The reported Q₁₀ values for methane production vary from 1.3 to 28 (van Hulzen et al., 1999; Segers, 1998). An increase in soil temperature increases the rate of organic matter decomposition by microorganisms in the soil, and lowers the redox potential (Tsutsuki and Ponnamperna, 1987), resulting in increased rate of CH₄ production.

The increase in CH₄ production with soil temperature means the projected global warming could enhance CH₄ production (Rath et al., 2002). Because of the uncertainties in the documented temperature response of CH₄ production, an understanding of the temperature feedback mechanisms remains poorly understood (Wang et al., 1999). Because of this uncertainty, most model studies have assumed Q₁₀ values of around 2 to 6 (van Hulzen, 1999; Xu et al., 2007; Huang et al., 1998; Huang et al., 2002; Yu et al., in press). Understanding the temperature - CH₄ production link is essential to accurately project future CH₄ emissions from rice paddies and possibly wetlands.

5.2 Aim and Objectives

The aim was to study the effect of temperature on CH₄ production, comparing production rates from both planted and unplanted microcosms under different

temperature treatments. The objective of this research was to determine the Q_{10} of CH_4 production under carefully controlled environmental conditions.

5.3 Material and Methods

We determined the rate of CH_4 production at different temperatures (20, 24, 28, 32 °C) using incubation experiments. The soil samples were extracted from both planted and unplanted tubs using sterilized plastic soil corers. The freshly extracted samples were quickly transferred into sterile whirl packs and homogenized before being put into specially designed Erlenmeyer flasks fitted with an airtight seals. The glass jars were equipped with insulated thermocouples and vent pipes with air tight valves.

These containers were immersed in the same water baths that were used to heat both the planted and unplanted tubs (Fig. 5.2). The jars were purged with 99.9 % N_2 before and immediately after placing a sample inside these containers. The first sample was immediately taken, and subsequently every 10 minutes thereafter. A total of four samples were collected in the experiments.

Each time, before a sample was withdrawn, the headspace gas inside the jar was homogenized thoroughly using a syringe and plunger. From each temperature treatment, two-10 mL vials of the headspace gas were collected from two jars, and taken to the laboratory analyzed for CH_4 on chromatograph equipped with FID.



Fig. 5.2: Picture of production jars mounted onto the water bath system. The jars are fitted with valves and thermocouples connected to microloggers (not shown in picture). Temperature was continuously monitored and recorded every 5 minutes during the growing season. The glass jars were purged with N_2 several times before each sample was collected.

The production rates for each of the temperatures were calculated as the slope of the plots of concentration versus time. The temperature coefficients of methane production were calculated from mean production rates at four temperature treatments.

5.4 Results and Discussion

5.4.1 Production Rates

Consistent with results from other studies (Cicerone and Shetter, 1981; Cicerone et al., 1983; Sass et al., 1991; Neue, 1993; Khalil et al., 2008), CH₄ production rates generally increased with time (DAT) over the growing season. The values were almost zero, two weeks after transplanting then increased on the third week. This delay in the production of CH₄ is thought to be a characteristic of the soil redox potential, pH, substrate availability, and temperature. For example the production of CH₄ have been found to commence hours after flooding in alkaline and calcareous soils, two to three weeks in neutral soils, and five or more weeks in acidic soils (Neue, 1993). The rates continued to increase (with highest rate occurring in highest temperature treatments), reaching a maximum between 96 and 100 DAT (Fig. 5.3). The general increase with DAT observed in all temperature treatments could be caused by several factors that include: population growth of bacteria and Archea following flooding, decay of residual plant material (organic substrate availability) and the growth of plant roots. Roots are thought to both positively and negatively contribute to CH₄ production: root oxygen transport suppresses CH₄ production, whereas root decay and root exudation promote CH₄ production (Sass et al., 1991; Segers, 1998).

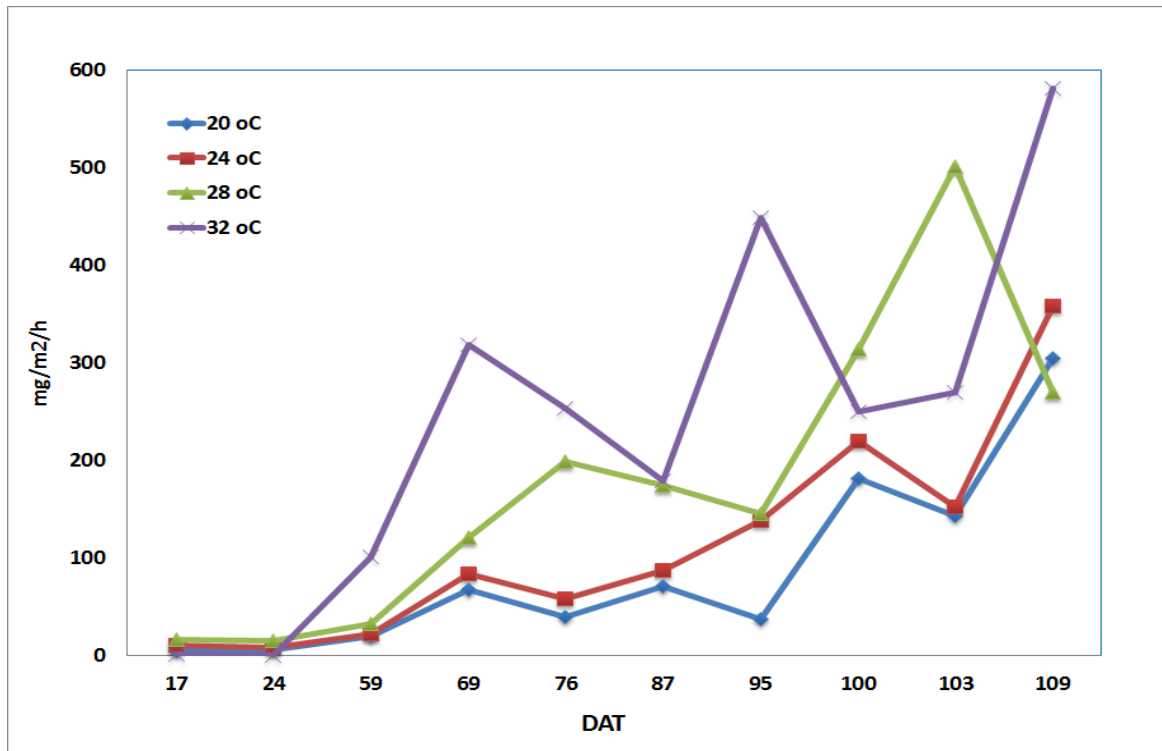


Fig. 5.3: CH₄ production rates, showing increases after 2 weeks following flooding. The highest production rates were associated with highest temperature after 17 days following transplanting (DAT).

The highest increase in CH₄ production occurred in the set-up with the highest temperature (32 °C). In a similar manner, the 32 °C temperature treatment had the highest production rate. The explanation for this observation is that immediately after flooding, increasing temperature enhances the reduction of Fe³⁺ and SO₄⁻ ions (van Hulzen et al., 1999) so that the beginning of CH₄ production critically depends on temperature (Conrad, 2002). The lowest temperature (20 °C) had the lowest production rate. Results are similar to studies by Aulakh et al. (2001) in which the presence of rice plants lowered the CH₄ concentrations in rice tubs, but had no net effect on the CH₄ production rates. Chin et al. (1999) observed that decreasing the incubation temperature of rice soil from 30 to 15 °C resulted in a decrease in the CH₄ production. Similarly,

Wang et al. (1999) found that CH₄ production rates that started to increase in the second or third week after flooding, reaching a maximum in the third and fourth week after flooding. It has been found that at temperatures higher than 30 °C, CH₄ is predominantly produced from H₂/CO₂, while acetate is no longer consumed and accumulated (Fey et al., 2001). Temperature also significantly affects the pathway of carbon flow since some microbial processes are more sensitive to temperature than others (Conrad, 2002).

When compared with CH₄ fluxes and pore water concentration trends, the flux, followed by pore water concentration trends, declined earlier than the production rates, with fluxes declining much earlier than the production rates, consistent with field experimental results reported in other studies (Khalil et al., 2008; Schütz et al., 1989). Temperature affects the growth of microbial populations, producing a non-linear response of CH₄ production to temperature. In addition, the availability and increased abundance of substrates strongly enhances the temperature response (Dunfield et al., 1993). The applicability of Arrhenius kinetics may be limited under conditions of changing substrate availability.

5.4.2 Temperature Dependence of CH₄ Production

The Q₁₀ of CH₄ production did not change with increasing DAT, varying from <1 and 8.5 (Fig. 5.4).

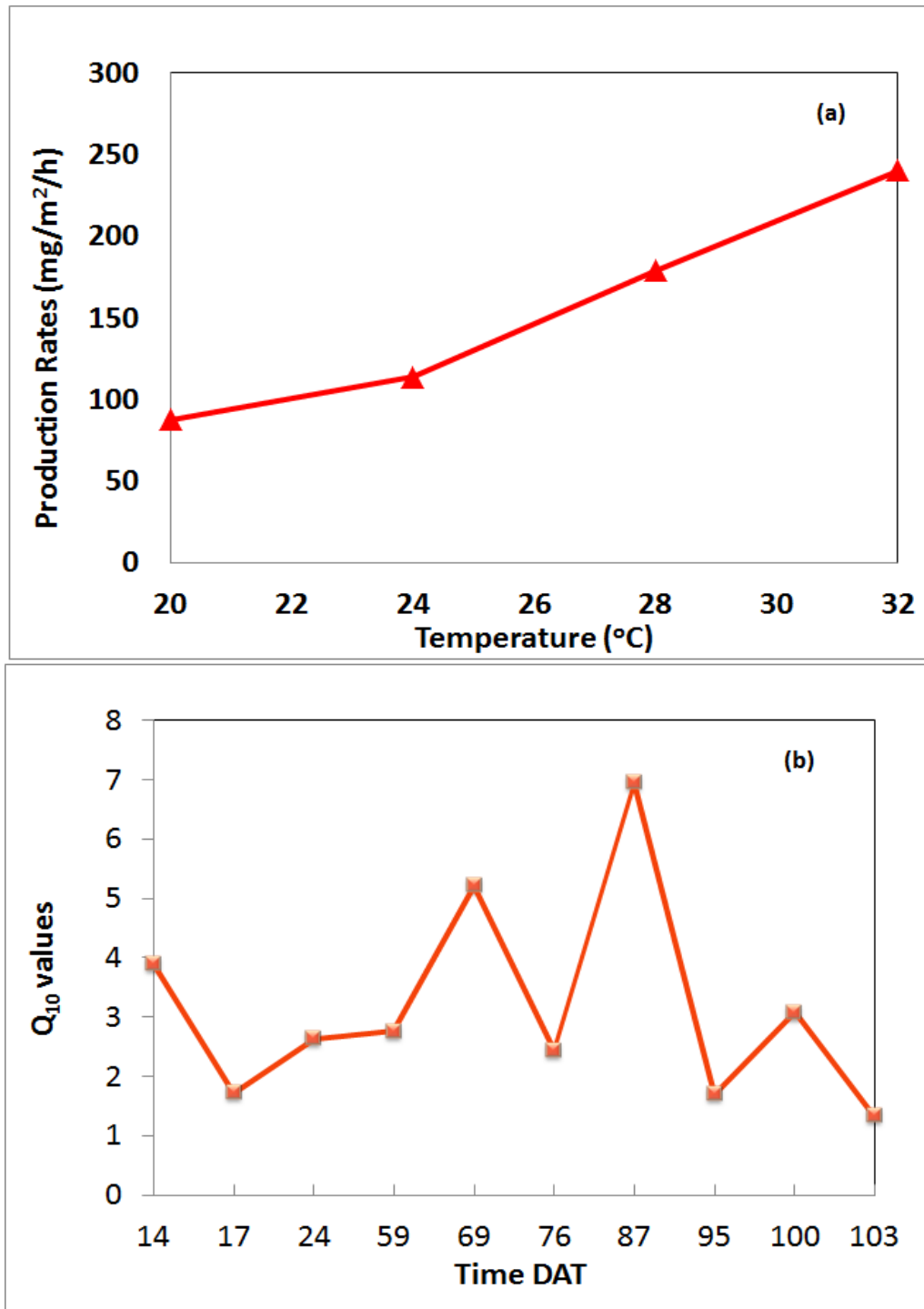


Fig. 5.4: (a) Average CH₄ production rates as a function of temperature, and (b) Q₁₀s of CH₄ production based on the incubation technique.

In other studies, Q_{10} values of CH_4 production varied from 1.5 to 3.6 (van Hulzen et al., 1999), and 1.1 to 5.5 (Rath et al., 2002). The seasonally averaged Q_{10} of production was found to be 2.6, with an upper and a lower limit of 2.1 and 3.3, respectively (90 % confidence level). At low temperatures, electron acceptors and methanogenic biomass activity limit methane production for a longer time leading to low methane production at low temperatures. Bacteria can use a limited number of substrates, of which acetate and hydrogen are considered the most important ones (Segers, 1998). Temperature also affects the diversity of methanogens in the soil. For example, Methanosarcinaceae, which utilize both H_2/CO_2 and acetate as the precursor to produce methane, have been found to be more dominant at higher temperature than Methanosaetaceae which only utilize acetate as the precursor for methane.

5.4.3 Estimation of CH_4 Oxidation rates

By considering the differences between seasonal production rates and fluxes, the seasonal fraction of CH_4 oxidized was determined based on the mass-balance approach. Values of oxidation rates varied increased from $50 \text{ mg/m}^2/\text{hr}$ to about $230 \text{ mg/m}^2/\text{hr}$, following a temperature increase of $20 - 32 \text{ }^\circ\text{C}$. Within the same range, the fraction of CH_4 oxidized increased from about 73 to 87 % (Fig. 5.5). The values are consistent with oxidation rates reported in Schütz et al. (1989). From this data, the calculated seasonal Q_{10} of oxidation varied between 2.8 (lower limit) to 4.5 (upper limit) with a seasonal average of 3.5 (90 % confidence limit).

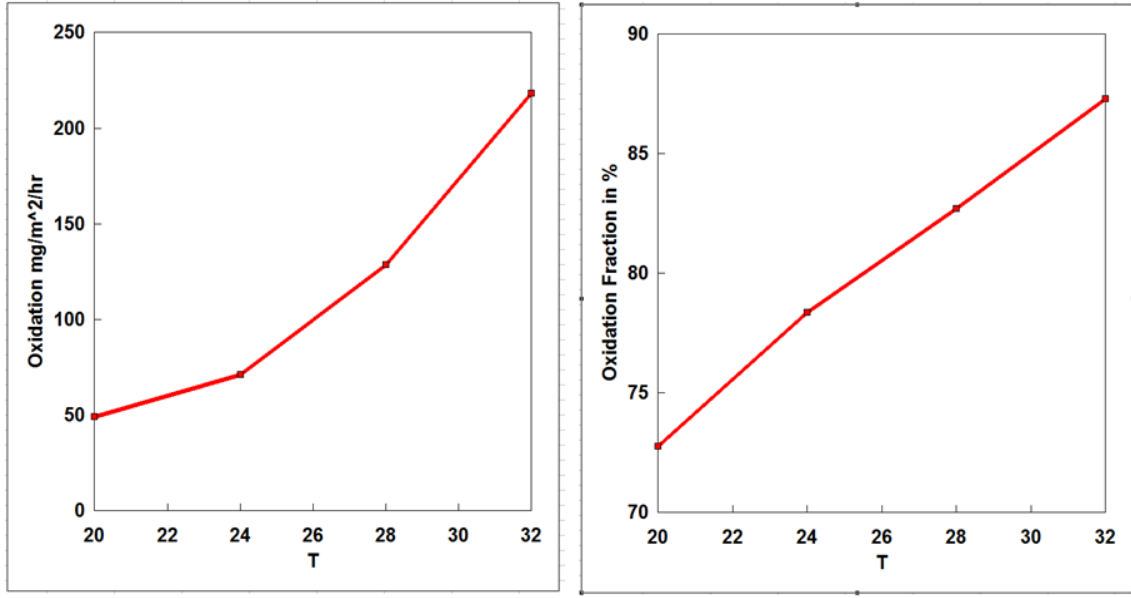


Fig. 5.5: CH₄ oxidation rates and fractions plotted against temperature (T, °C).

5.4.4 Estimation of Q₁₀ of Transport

Based on the Q₁₀s production, flux and oxidation, the Q₁₀ of transport was calculated using the following approach:

$$Q_{10}(\text{flux}) = Q_{10}(\text{production}) \times \frac{Q_{10}(\text{transport})}{Q_{10}(\text{oxidation})}$$

The Q₁₀ of transport varied from 1.0 (lower limit) to 7.0 (upper limit), with a seasonally averaged value of 2.4.

5.5 Conclusion

The effect of temperature on CH₄ production under continuously flooded rice ecosystems was successfully determined in this research. The rate of CH₄ production was strongly dependent on soil temperature, with a seasonally averaged Q₁₀ value of 2.6.

The seasonally averaged Q_{10} of oxidation and transport were 3.5 and 2.4. The variations, as well as the values found in this research, were less than those reported in literature cited herein, in which values of up to 28 have been presented. Within the range of temperature considered in this research, elevating soil temperature enhances CH_4 production in continuously flooded rice ecosystems, and possibly wetlands.

Chapter 6

Quantifying Temperature Effect on Methane Oxidation in Rice Ecosystems using Stable Carbon and Hydrogen Isotope Ratios

6.0 Summary

The sensitivity of rhizospheric CH₄ oxidation to elevated soil temperatures was determined using naturally occurring stable hydrogen and carbon isotopic abundances in water bath temperature-regulated rice microcosms described earlier. CH₄ fluxes, below- and aboveground carbon ($\delta^{13}\text{C}$) and hydrogen (δD) isotopic composition of CH₄ was measured using continuous-flow gas chromatography isotope ratio mass spectrometry in Dr. Andrew Rice's laboratory at Portland State University. The belowground pore water samples were collected from each treatment weekly and CH₄ was extracted into N₂ headspace. Isotopic measurements showed that the mean above-ground $\delta^{13}\text{C}$ values ranged between -60.4 and -53.0 ‰, with an average of -56.7 ‰ relative to V-PDB, while δD values ranged between -341.9 and -273.3 ‰ relative to V-SMOW. The ranges for the below-ground isotopic composition were -52.6 to -45.6 ‰ and -348.3 to -246.1 ‰ for $\delta^{13}\text{C}$ and δD , respectively. CH₄ flux ranged from near zero to 30-60 mg/m²/hr in mid-season corresponding to a rise in pore water CH₄ to 8-12 mg/L. These results were integrated and interpreted using an empirically-driven concentration and isotope model to understand CH₄ dynamics and to examine the effect of temperature on mechanisms that control CH₄ emissions. The Q₁₀ of methane oxidation was found to be 1-2 and 1.4-2.4 from the $\delta^{13}\text{C}$ and δD values, respectively. This study showed that temperature plays a less important role in CH₄ oxidation in rice

paddies compared with production (reported in Chapters 3 and 5) where the average Q_{10} s were 4.6 and 3.1, respectively. Thus the change in the CH_4 oxidation efficiency due to elevated soil temperature would not be enough to offset the effect of temperature on CH_4 production and emissions, thereby increasing the likelihood of increased CH_4 emissions from rice agriculture under future global warming.

6.1 Introduction

Rice paddies represent a sizable anthropogenic source of CH_4 emissions into the atmosphere. Rhizospheric oxidation is a major sink of CH_4 in rice ecosystems. Without this process, CH_4 emissions from rice agriculture would be perhaps an order of magnitude higher than the present rates. Yet the impact of global warming on CH_4 oxidation, and on the global CH_4 atmospheric budget remains poorly understood. Globally, CH_4 oxidation is a sink for CH_4 and is important in reducing the CH_4 emissions into the atmosphere (Hanson and Hanson, 1996; Born et al., 1990). Two principal mechanisms involved are: (1) reactions in the atmosphere, and (2) microbial CH_4 oxidation by bacteria in soils. The net flux from rice and wetland ecosystems is a residue of the production and microbial oxidation process. Microbial oxidation of the CH_4 produced in the soil is a large fraction of the soil methane pool. As these microbial processes change, the net flux into the atmosphere from these ecosystems will also change. Thus soil microbial CH_4 oxidation is considered a key determinant of the rate of change of atmospheric CH_4 concentration (Wang and Ineson, 2003).

It is estimated that up to 90 % of the CH₄ produced in flooded rice paddies is oxidized microbially adjacent to the zone of production before reaching the atmosphere (Schütz et al., 1989). Two main aerobic areas contribute to CH₄ oxidation (Wang et al., 1997): (I) the oxidized soil surface layer and, (II) the root rhizosphere. The process is mediated by an unidentified community of aerobic methanotrophic bacteria (Conrad, 1996). These aerobic methanotrophs, part of a large group of microorganisms called methylotrophs, reside in the rhizosphere where they use O₂ from the atmosphere that diffuses into the soil or the root zone through plants to oxidize CH₄. An aerobic surface layer develops around the plant roots. The thickness of this layer is determined by the net consumption and transport rates of O₂ supply through plants aerenchyma and intercellular gas space system (Chanton et al., 1992, Nouchi et al., 1990) by diffusion or by mass flow from atmosphere into the root zone. Thus the rate of O₂ supply to the rhizosphere is a decisive factor in the magnitude of CH₄ emission. Hence, rhizospheric CH₄ oxidation is an important internal sink for CH₄ produced in the flooded soils, consuming up to 90 % of CH₄ production. The presence of O₂ in the rhizosphere limits CH₄ production and enhances oxidation (Wang et al., 1997). This supply of O₂ to the rhizosphere enhances the activity of aerobic methanogens leading to higher CH₄ oxidation efficiency. Thus, under these conditions, both plant-mediated CH₄ emission and the percentage of CH₄ oxidized increase. It is thought that approximately 580 Tg y⁻¹ of CH₄ is produced in flooded rice paddies, but only about 60-100 Tg y⁻¹ escapes into the atmosphere (Hanson and Hanson, 1996). With projected global warming, persistent shifts in soil temperature could significantly alter the atmospheric CH₄ budget.

6.1.1 Other Factors Affecting CH₄ Oxidation in Flooded Soils

The elements influencing CH₄ oxidation in flooded soils depend on factors that affect methanotrophic activities such as O₂, temperature, CH₄ availability and the mode of CH₄ transport from the production zone to the atmosphere. It is estimated that the optimum temperature for CH₄ oxidation ranges between 25 and 30 °C (Whalen and Reeburgh, 1996). In the same studies, CH₄ oxidation has been found to be correlated to temperatures.

The nutritional status of a plant affects its isotopic discrimination, such that well-nourished plants exhibit more positive δ¹³C values by 1-2 ‰ than plants with nitrogen (N) or potassium deficiency (O'Leary, 1981). The kinetics of CH₄ oxidation in soils is complex and its dependence on soil N status remain an area of debate (Reay and Nedwell, 2004). The availability and quantity of nitrogen compounds have been found to have an effect on the oxidation of CH₄. Several studies have demonstrated that elevated soil N, particularly in the form of NH₄⁺, may reduce CH₄ oxidation rates. In particular, increased inhibition of CH₄ oxidation by NO₃⁻ has been observed at low CH₄ concentrations (Reay and Nedwell, 2004).

Another factor that influences oxidation is the limited supply of gaseous substrates to aerobic methanotrophs. For example, the distribution of aerobic methanotrophs depends on the flow of CH₄ from the production (anaerobic) zone and the transport of O₂ from the atmosphere into the soil (Hanson and Hanson, 1996). In planted flooded soils, O₂ is transported into the root zone via the plants. Another important factor may be the

consumption of O₂ by other microorganisms before it used by aerobic methanotrophs, particularly in soils with high degradable carbon content. The interactive effects of these regulators are uncertain under different environmental conditions.

6.1.2 Quantifying CH₄ oxidation

Several approaches have been used to quantify CH₄ oxidation in the rhizosphere: (I) mass balance calculations based on known concentration of CH₄ (Reeburgh, 1976; Rudd and Taylor, 1980; Powelson et al., 2007; Khalil et al., 2008), (II) soil core incubations (Rudd and Taylor 1980), (III) inhibitors such as methyl fluoride (Popp et al., 2000), (IV) inhibition of methanotrophy by using N₂, and (V) measurement of ¹³C-isotopic signatures of the produced CH₄ and CO₂ (Rudd and Taylor, 1980; Chanton et al., 2008a; Powelson et al., 2007; Venkiteswaran and Schiff, 2005; Groot et al., 2003; Conrad et al., 2002; Gerard and Chanton, 1993; Epp and Chanton, 1993). The mass-balance method requires CH₄ and CO₂ emission and production measurements. The rate of CH₄ oxidation per unit area is then calculated as a difference between production and emission. Because of the difficulty in accurately quantifying diffusion coefficients, the mass balance technique is associated with uncertainties (Chanton et al., 2009). Physical characteristics of an environment are not preserved in incubation experiments. For example the incubation technique is affected by contamination during sampling, distortion of the physical structure of the soil samples, changes in microbial communities and the changes of environmental conditions in the laboratory such as temperature.

Of all the methods above, isotopic analysis of soil CH₄ is a direct and non-invasive technique used to monitor the CH₄ cycle processes at a finer scale than is possible by measurement of surface CH₄ emissions only (Whiticar et al., 1986). The technique has found extensive use in the assessment of biogeochemical processes such as CH₄ production and oxidation in landfills, terrestrial and marine environments (Nakagawa et al., 2002). The method is based on determining the proportion (fraction) of CH₄ oxidized from the differences between stable and non-destructive hydrogen and carbon isotopic ratios. The technique is widely used to quantify CH₄ oxidation in rice paddies, temperate soils and natural wetlands because of its advantages over techniques mentioned above: (I) it has a relatively sensitive, which makes it a robust approach for in-situ measurements (Chanton et al., 2008b; Boerjesson et al., 2007; Bodelier et al., 2000), and (II) is non-invasive, leaving the rhizosphere intact compared to incubation or inhibition techniques.

Typically, natural biogenic CH₄ has a carbon isotopic composition below -75 ‰ (Tab. 6.1), but due to oxidation, CH₄ may exhibit ¹³C enriched values of -30 to -50 ‰. These methanotrophic bacteria preferentially consume ¹²CH₄ and discriminate against the heavier ¹³CH₄ (Chanton and Liptay, 2000; De Visscher et al., 2004; Happell et al., 1994; Whiticar 1999) leaving residual CH₄ enriched in ¹³CH₄ (O'Leary et al., 1988; Reeburgh et al., 1993). The resultant effect is a change in the isotope composition when CH₄ is oxidized, thereby altering the isotope ratio. Because of this property, CH₄

oxidation is estimated by the degree of change between the $\delta^{13}\text{C}$ content of CH_4 emitted relative to the $\delta^{13}\text{C}$ of CH_4 in the anoxic zone.

Table 6.1. Overview of typical isotope measurements on CH₄ from rice paddies.*

Location	$\delta^{13}\text{C}$	δD	Seasonal Coverage	Sampling Method/ Sample Type	Reference
California	-68 to -66	...	3 weeks before harvest	nocturnal inversion	[Stevens and Engelkemeir, 1988]
Kenya	-63 to -57	...	days 44, 72, 100, 128, 156 (after sowing)	static chamber	[Tyler et al., 1988]
Louisiana	-66 to -60	...	May-June	...	[Wahlen et al., 1989]
Italy	-68 to -63	-339 to -319	days 85-88 (after flooding)	static chamber	[Bergamaschi, 1990]
Japan	-68 to -48	...	throughout the season (maximum interval 17 days)	gas bubbles	[Uzaki et al., 1991]
...	-65 to -61	-310 to -290	[Wahlen, 1993]
Japan	-71 to -56	...	days 22, 51, 71, 93, 107 (1990)	static chamber	[Tyler et al., 1994]
	-72 to -58	...	days 38, 72, 100 (1991) (after flooding)		
Louisiana	-66 to -61	...	days 84-89 (after seeding)	CO ₂ controlled chamber	[Chanton et al., 1997]
	-61 to -57	-325 to -300		gas bubbles	
	-56 to -50			lacunal	
China	-71 to -52	-352 to -311	every 7 days	static chamber	[Bergamaschi, 1997]
	-71 to -58	-358 to -343	every 1-4 weeks	gas bubbles	

*Adapted from Bergamaschi [1997]

The fractionation due to microbial oxidation is affected by different processes such as transport, temperature (Chanton and Liptay, 2000), methanotrophic activity and CH₄ availability (Templeton et al., 2006), and enzymatic reactions or a mixture of all.

6.1.4 Quantification of CH₄ Oxidation

The carbon isotopic composition is expressed in the standard δ notation in per mil. (Gonfiantini et al., 1995):

$$\delta^{13}\text{C}(\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

Where R_{sample} is the carbon-13 abundance ratio given by ¹³CH₄/¹²CH₄ ratio of the sample.

Since absolute values of ¹³C/¹²C are difficult to obtain, the values are reported relative to Vienna Peedee Belemnite (VPDB) (O’Leary, 1981; Gonfiantini et al., 1995). R_{standard} is the ¹³CH₄/¹²CH₄ ratio for V-PDB. Negative $\delta^{13}\text{C}$ values indicate that depletion of the ¹³C isotope is taking place relative to the carbonate standard. In a similar way, just recently, the use of hydrogen isotopes (δD) has been used in trying to understand the biogeochemistry of individual CH₄ sources (Wahlen, 1994; Quay et al., 1999; Rice et al. 2009; Tyler et al., 1997). The hydrogen isotopic composition, δD , is calculated in the same way using the D/H ratio for standard mean (0.0001558) for R_{standard} (Gonfiantini et al., 1995). The percentage fraction of oxidized CH₄ (oxidation efficiency) is determined as a function of isotopic belowground CH₄ (δ_i), emitted CH₄ (δ_f), and the kinetic isotope effect (KIE) during oxidation (α):

$$F = \frac{\delta_i - \delta_f}{(\alpha - 1)(\delta_f + 1000)}$$

Where δ_i is the $\delta^{13}\text{C}$ (or δD) value in the anoxic zone, δ_f is the $\delta^{13}\text{C}$ (or δD) value of emitted CH_4 , α is the fractionation factor, α for microbial oxidation and α_{trans} is the fractionation factor associated with gas transport. The extent of bacterial oxidation is estimated by α , which is the difference between the production and the residual CH_4 . Typical values of α associated with $\delta^{13}\text{C}$ and δD values are presented in Table 6.2.

Table 6.2: Overview of the kinetic isotopic effects during CH₄ oxidation by aerobic bacteria*

Study	α^p_{ox}	α^c_{ox}	Reference
Laboratory enrichment cultures of methanotrophic bacteria	---	1.011	<i>Silverman and Oyama</i> [1968]
	---	1.016	<i>Lebedew et al.</i> [1969]
	---	1.011 - 1.039	<i>Zyakun et al.</i> [1988]
	1.103 - 1.311	1.0132 - 1.025	<i>Coleman et al.</i> [1981]
	---	1.0052 - 1.0313	<i>Barker and Fritz</i> [1981]
Landfill cover soils			
Germany	1.044 ± 0.020	1.008 ± 0.003	<i>Bergamaschi and Harris</i> [1995]
Europe	1.039 ± 0.026	1.008 ± 0.004	<i>Bergamaschi et al.</i> [1998]
NE United States	1.046 ± 0.016	1.022 ± 0.008	<i>Liptay et al.</i> [1998]
Swamp floodwaters	1.050 - 1.129	1.003 - 1.021	<i>Happell et al.</i> [1994]
Soils			
Boreal forest soils in situ (Alaska)	---	1.022 - 1.025	<i>Reeburgh et al.</i> [1997]
Native grassland soils (Washington)	1.136 - 1.209	1.0121 - 1.0183	<i>Snover and Quay</i> [2000]
Temperate forest soils (Washington)	1.094 - 1.128	1.0165 - 1.0177	<i>Snover and Quay</i> [2000]

*Adapted from *Snover and Quay* [2000]

Typically, values of α_{ox} have been found to be dependent on both temperature (Coleman et al. 1981; King et al., 1989) and CH_4 concentrations, with values lying in the range 1.022 to 1.050 for temperatures between 15°C and 26°C (Cabral et al., 2010; Tyler et al., 1997; Reeburgh et., 1997). Various models have been developed that relate α_{ox} and soil temperature (Fig. 6.1).

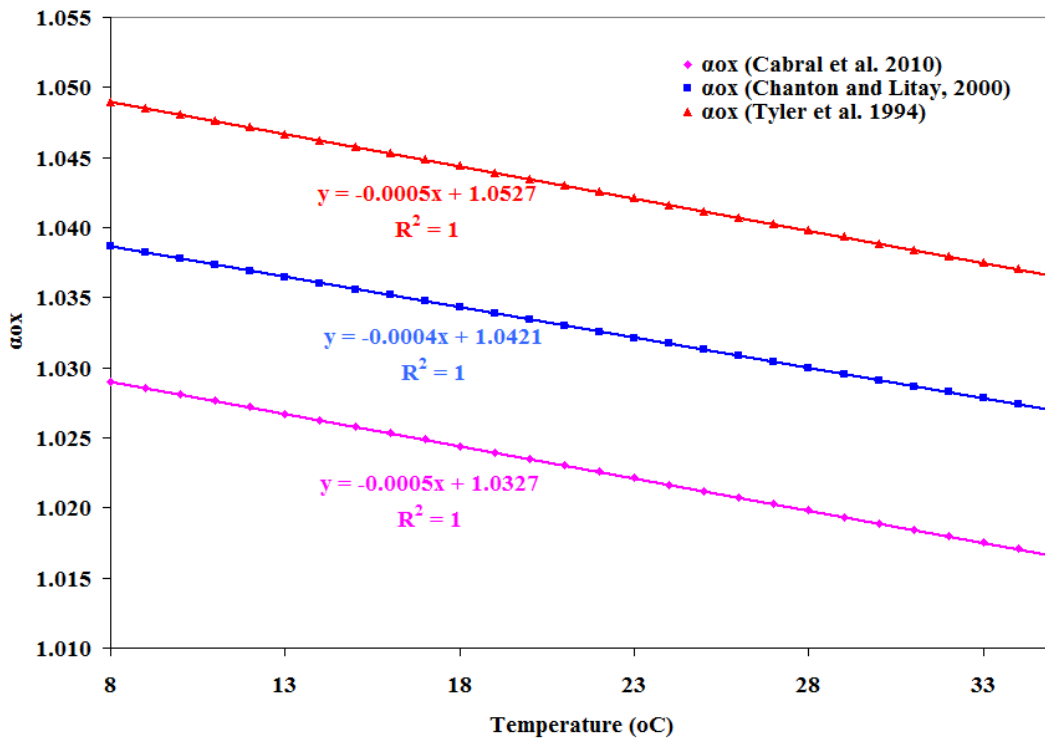


Fig. 6.1: Typical models proposed for the variation of α_{ox} with temperature. There is generally an agreement between the slopes, but with significantly different intercepts.

6.1.5 Isotope Fractionation by Transport

Three main modes of CH_4 transport from flooded rice paddies (Schutz et al., 1991) associated with ^{13}C isotopic fractionations (Chanton, 2004) are: diffusion, ebullition and

plant-mediated transport. The relative importance of these three pathways depends on organic loading, changes in temperature and plant density. For example, in bare plots (controls), isotope fractionation of CH₄ during transport is mostly due to molecular diffusion in the gas phase and equilibrium partitioning between gas and water. Bubble ebullition across the air-water interface and bulk flow are thought to contribute little or no isotopic fractionation (Chanton, 2004). In rice planted tubs, CH₄ transport is predominantly a combination of convective bulk flow (in daylight) and molecular diffusion (at night). The presence of plants also affects the stable isotope distribution of CH₄ in both fluxes and pore water through plant-mediated transport mechanisms and aerobic oxidation associated with the roots (Schutz et al., 1989; Chanton and Dacey, 1991). For example, diffusion through plant aerenchyma results in significantly greater rates of transport for ¹²CH₄ relative to ¹³CH₄ resulting in the enrichment of ¹³CH₄ in plant aerenchyma and in the rhizosphere (Chanton, 2004).

While during the day, pressurized through-flow (active transport) is the main mode of gas transport, with little isotopic fractionation of CH₄ (Chanton, 2004), the main transport mechanism at night is diffusion (passive transport), which result in mass-dependent fractionation of emitted CH₄ (Chanton and Whiting, 1996). The presence of diffusion results in the release of depleted CH₄ in fluxes than in pore water (Chaser et al., 2000). Fractionation in rice plants differs significantly from day to night as molecular diffusion is more important during night time.

In comparison to bare flooded soils, emissions from flooded soils planted with rice generally release about 9 times more CH₄, resulting in a reduction of CH₄ partial pressures within the flooded soil, thereby causing a reduction in dissolved CH₄ pore water concentrations and CH₄ bubbles (Chanton, 2004). The result is a dominant plant-mediated transport in rice planted tubs and bubble ebullition and diffusion in non-rice planted tubs.

6.2 Aim and Objectives

The major aim of this research was to determine how CH₄ oxidation in rice ecosystems will be affected by elevated soil temperatures under global warming. Three objectives were set: (I) determine stable hydrogen and isotopic compositions of CH₄ under different soil temperatures; (II) characterize seasonal patterns of CH₄ oxidation, and (III) use $\delta^{13}\text{C}$ and δD to calculate fraction and Q_{10} of CH₄ oxidation in rice ecosystems.

6.3 Materials and Methods

6.3.1 Site Characteristics

The study was performed in a climate-controlled greenhouse experiment as previously stated in Chapter 2. The rice plots were continuously flooded immediately after the rice seedlings were transplanted. These conditions were consistently maintained throughout the sampling period to maintain uniformity in environmental conditions. The fraction of oxidation was determined using in-situ stable isotope tracing technique (Chanton et al.,

1997; Tyler et al., 1997). The method makes use of naturally occurring carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) between above- and below-ground was used in this research.

6.3.2 Sampling Techniques

Three types of samples were collected throughout the season. Gas samples for flux measurements were collected from the rice tubs using static chambers as outlined in Chapter 3. Each time the samples above were collected, two 60 mL samples were withdrawn after 30 minutes using syringes for isotopic analysis. The 60 mL headspace gas samples were stored in pre-evacuated 30mL glass vials with non-reactive CH_4 stoppers (Bellco Co. USA) and stored at laboratory temperature for analysis.

Water samples were collected on a weekly basis using plastic peepers that were connected to small perforated cylinders placed at approximately 5, 10, 15 and 20 cm below the soil surface. The cylinders were placed between the rice rows or in the unplanted tubs (controls). Before pore water sample was collection, about 10 mL were flushed out of the peepers, and left for about 30 minutes to equilibrate. About 20 mL of pore water were withdrawn using syringes and taken to the laboratory for analysis. The headspace gas after mixing with 99.9 % N_2 was dried and stored into evacuated 20 mL glass vials.

6.3.3 Isotopic Measurements

The hydrogen and carbon isotope ratios in the samples were determined using continuous-flow gas chromatography isotope ratio mass spectrometry. For pore water samples where CH₄ concentrations were high, extracted samples were injected onto a Pava Plot on column via a 6-point valve with a 100 µl sample loop. Above-ground flux samples were enriched prior to analysis by trapping CH₄ at -125 ± 5 °C for several minutes. The resulting sample was then focused on a loop of the analytical column immersed in liquid nitrogen. Following the procedure described in Rice et al. (2001; 2010), the sample was warmed and released onto the separation column for analysis. In all analyses, elutes from the GC column, CH₄ was converted to either CO₂ (for δ¹³C) or H₂ (for δD) in a high temperature furnace. Following high temperature conversion, samples were introduced into a Thermo Scientific Delta V isotope ratio mass spectrometer in a viscous flow of helium. Isotope ratios were determined by integrating peaks of mass /charge: 44.45 (for δ¹³C) or 2.3 (for δD). The isotope ratios were determined with reference to the standards V-PDB (for δ¹³C) and V-SMOW (for δD).

To calculate the fraction of CH₄ oxidized, the background isotopic composition was accounted for when calculating the isotope ratios (δ_E) for CH₄ emissions:

$$\delta_E = \frac{c_F \delta_F - c_I \delta_I}{c_F - c_I}$$

Where δ_I and C_I are the δ¹³C or δD and concentration for the initial gas sample and δ_F and C_F refer to the δ¹³C or δD and concentration for the final sample.

6.3.4 Determination of Fraction of CH₄ Oxidized

From isotopic studies of flux samples from vegetated wetlands, emitted CH₄ has been found to be 5-15 ‰ more depleted in δ¹³C than in unplanted samples (O’Leary et al., 1988; O’Leary, 1981). Tyler et al. (1997) and Craig noted in O’Leary (1981) found that CH₄ was depleted by -12 ‰ for rice plants. With this value, the above-ground isotopic composition was adjusted for transport fractionation in order to get the below-ground values. Following the protocol in Tyler et al. (1997), the fractional oxidation was calculated as a function of isotopic below-ground CH₄ emitted CH₄ and α values from Snover and Quay (2000).

6.4 Results and Discussion

6.4.1 Carbon Isotopic Composition

Large temporal variations in CH₄ fluxes, δ¹³C and δD were observed during the middle of the growing season, in resemblance to those reported in Tyler et al. (1997). The mean above-ground δ¹³C values were between -60.4 and -53.0 ‰, with an average of -56.7 ‰ relative to V-PDB. These compare well to reported mean values of between -66 and -50 ‰ from temperate rice paddies (Marik et al., 2002; Bergamaschi, 1997). Consistent with results from Indian rice paddies (Rao et al., 2008), the emitted CH₄ at the start of the growing season was relatively enriched in ¹³C, then depleted during the middle part of the season, and finally enriched again towards the end of growing season (Fig. 6.2). These seasonal changes are thought to be due to changes in fractionation associated

with CH₄ production, oxidation and transport (Rao et al., 2008) due to plant roots growth and decay.

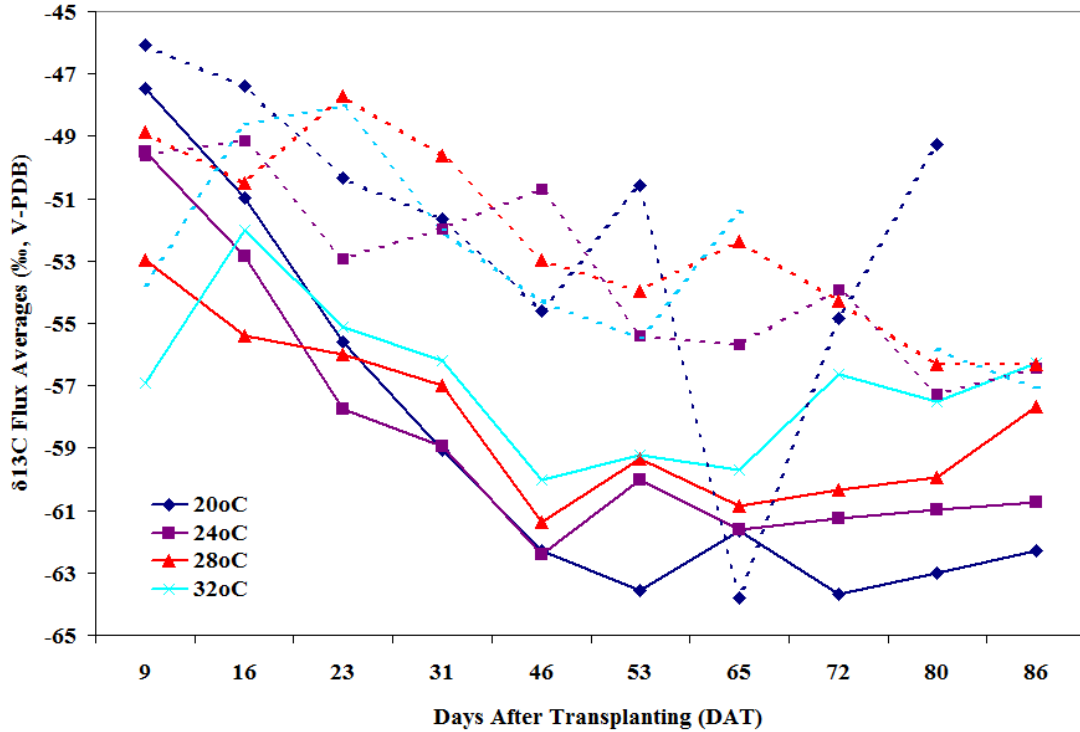


Fig. 6.2: Seasonal variation of $\delta^{13}\text{C}$ of CH_4 emitted from rice planted tubs (solid lines) and unplanted tubs (broken lines) for different temperature treatments. In all treatments, the samples from planted tubs were more depleted during the middle part of the growing season than at the start. That feature was not evident in unplanted (control), except in one particular treatment (20 °C) in which the trend was similar to those in planted tubs.

The isotopic composition of pore water CH₄ at 10 cm depth ranged from -52.6 to -45.6 ‰. Seasonal trends were similar in planted and unplanted tubs, with no distinct differences between different temperature treatments (Fig. 6.3).

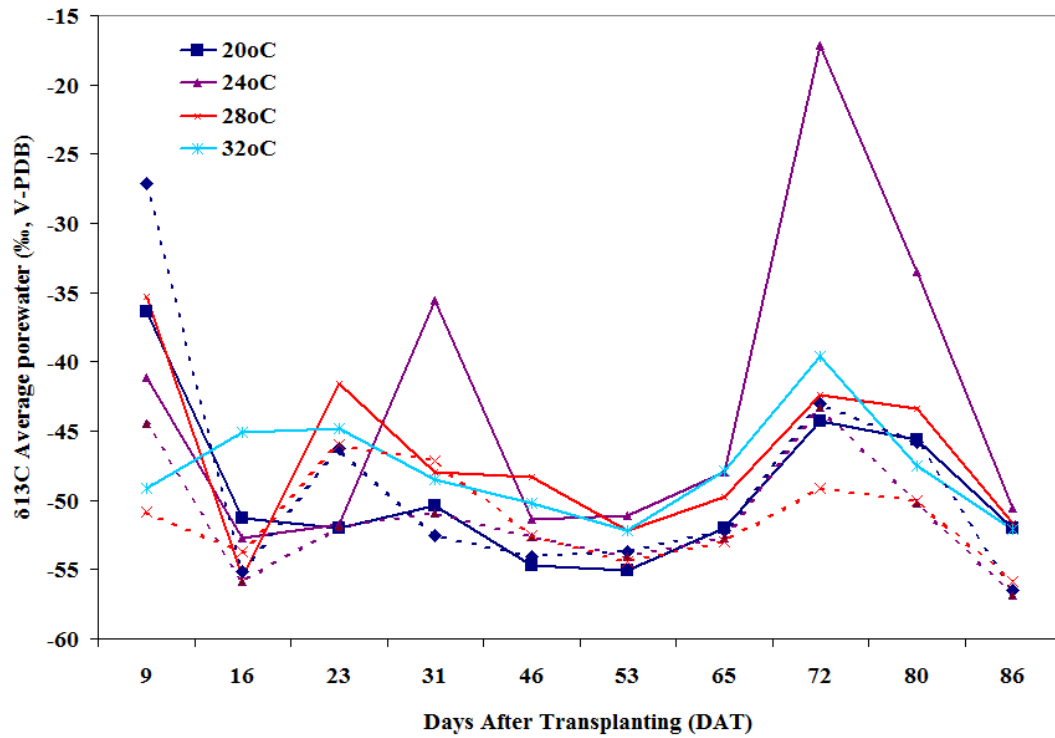


Fig. 6.3: Seasonal variation of $\delta^{13}\text{C}$ of CH_4 in pore water from planted tubs (solid lines) and unplanted tubs (broken lines) for different temperature treatments.

Aboveground δD values ranged between -341.9 and -273.3 ‰ (mean -317.0 ‰), while the belowground isotopic compositions were -348.3 to -246.1 ‰ (mean -308.3 ‰) for δD , respectively. Large temporal variability is observed in both isotope tracers and is not well correlated with emitted isotopic composition. The variation is comparable to aboveground isotopic composition of between -352 and -311 ‰ V-SMOW (mean -335.7 ± 10 ‰) as reported in other studies (Bergamaschi, 1997). Three factors are responsible for these variations: (I) changes in the CH_4 formation pathway, (II) changes in isotopic composition of methanogenic precursors, and (III) isotopic fractionation due to CH_4 oxidation.

Similar trend toward depleted δD as DAT increased was observed. For example, the isotopic composition of emitted CH_4 in planted tubs ranged from -64 ‰ to -47 ‰ $\delta^{13}C$ versus V-PDB and -354 ‰ to -146 ‰ δD versus V-SMOW with a trend towards more depleted values as the season progressed.

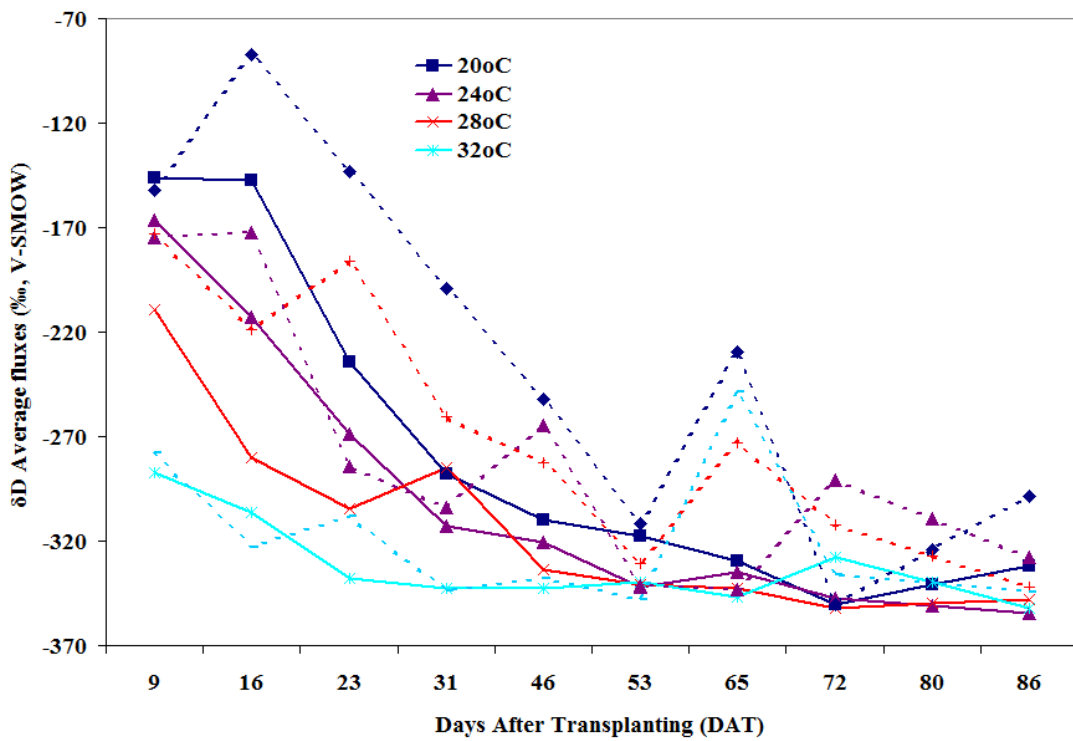


Fig. 6.4: Time series of δD of CH_4 showing hydrogen isotopic composition in rice planted tubs (solid lines) and unplanted (control) tubs (broken lines) for different temperature treatments. Generally, the value are enriched at the start, and then depleted till the end of sampling period.

The isotopic composition of pore water CH₄ at 10 cm depth ranged from -380 to -153 ‰ δD (Fig. 6.5). Large temporal variability was observed in both isotope tracers and was not well correlated with emitted isotopic composition.

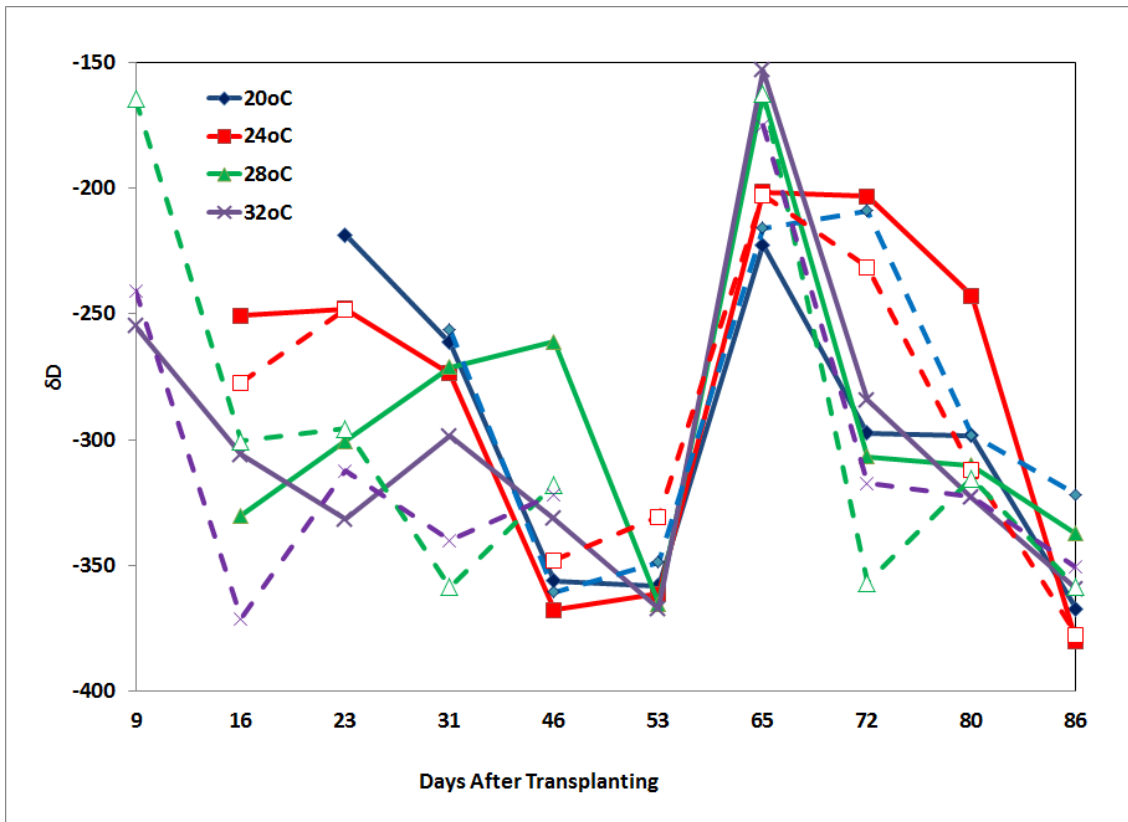


Fig. 6.5: Time series of pore water δD of CH₄ showing hydrogen isotopic composition in rice planted tubs (solid lines) and unplanted (control) tubs (broken lines) for different temperature treatments.

By considering the results of classification of bacteria based on $\delta^{13}C-CH_4$ and $\delta D-CH_4$ information (Fig. 6.6), the range of results also indicate that the main methanogenic pathway of CH₄ formation in this study was predominantly aceticlastic methanogenesis (acetate fermentation), which, respectively, is associated with isotopic composition of -

65 to -50 ‰ V-PDB (Grossman, 2002; Whitcar, 1999) and -400 to -250 ‰ (V-SMOW) for $\delta^{13}\text{C}$ and δD (Bergamaschi, 1997).

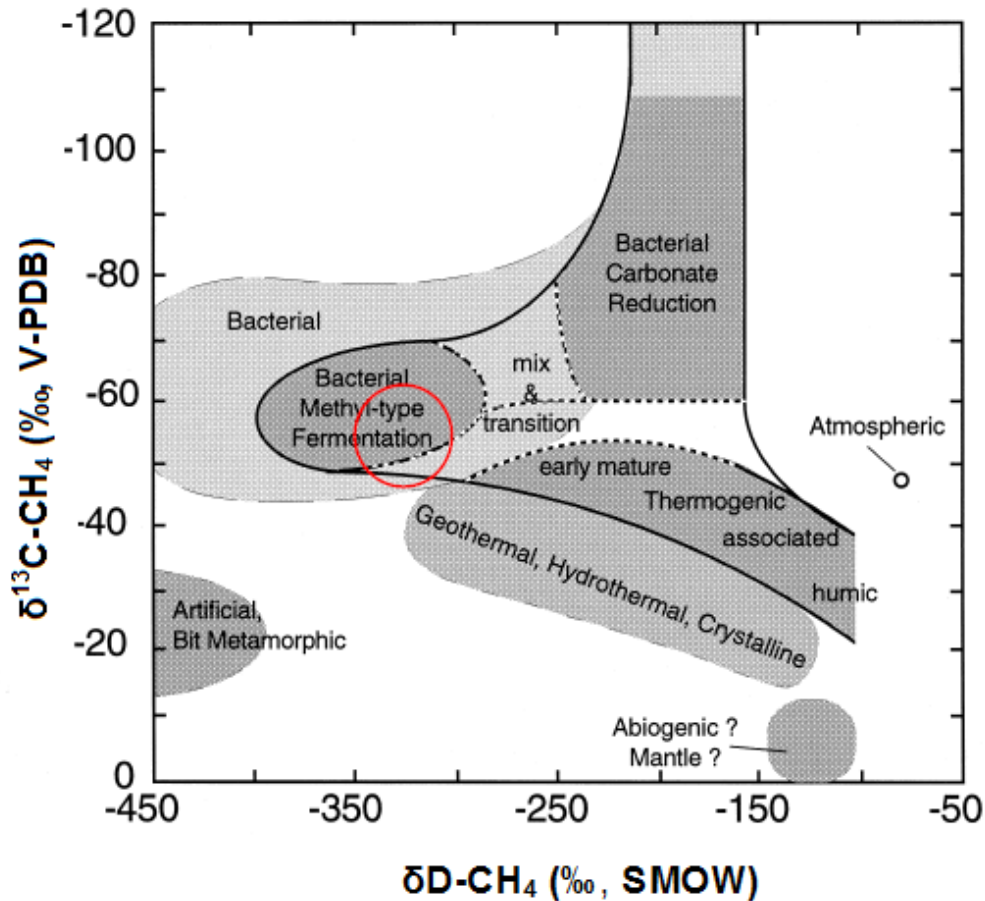


Fig. 6.6: Classification of bacterial and thermogenic natural gas by the combination of $\delta^{13}\text{C-CH}_4$ and $\delta\text{D-CH}_4$ information (Adapted from Whitcar 1999). Compared with $\delta^{13}\text{C-CH}_4$ and $\delta\text{D-CH}_4$ found in this research, results showed that the predominant source of CH_4 was acetate (red circle), and not through CO_2 reduction.

The other methanogenic pathway for CH_4 formation (CO_2 reduction) is generally more depleted in $\delta^{13}\text{C}$ (-110 to -60 ‰ V-PDB) and more enriched in δD (-200 to -110 ‰ V-SMOW) (Bergamaschi, 1997; Whitcar, 1999), and thus, was not the major pathway in this study.

Emitted values from planted tubs were substantially depleted in $\delta^{13}\text{C}$ (~6 ‰) and δD (~30 ‰) relative to unplanted (control) tubs, a difference which is understood to result from isotopic fractionation during diffusive (and potentially effusive) transport from the anaerobic zone through the rice plant. Seasonal mean values of $\delta^{13}\text{C}$ were higher at higher temperatures (-59.0 ‰, -58.6 ‰, -58.0 ‰, -57.0 ‰, 20-32 °C) and δD was lower at higher temperatures (-272 ‰, -296 ‰, -311‰, -331‰). This effect is not yet well understood.

6.4.2 Relationships between $\delta^{13}\text{C}$ and δD in Emitted and Dissolved CH_4

Very high positive regression coefficients ($r^2 = 0.760$ to 0.957) were found between $\delta^{13}\text{C}$ and δD in temperature treatments 20-28 °C. At 32 °C, the regression coefficient and slope were very low ($r^2 = 0.199$; slope = 0.2039). In these three treatments, the regression curves, the slopes were around 14 (Fig. 6.6). Consistently, Coleman et al. (1981) found change in the δD value of CH_4 (which has been partially oxidized by bacteria) is 8-14 times greater than the change in the $\delta^{13}\text{C}$ value.

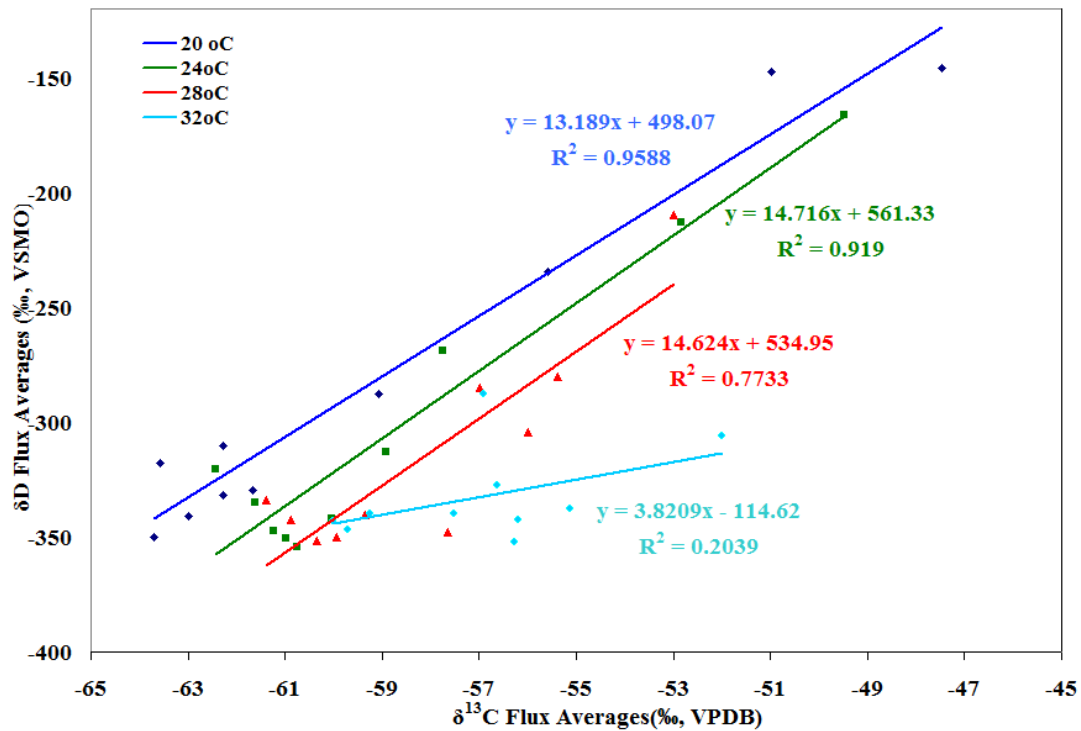


Fig. 6.7: Effect of temperature on the relationship between isotopic composition, $\delta^{13}\text{C}$ and δD values in the air samples from planted tubs.

In general, all regression coefficients were positive, but were higher in lower temperature treatments. Similarly, positive correlations between $\delta^{13}\text{C}$ and δD ($r = 0.51$) were found from rice fields experiments in China (Bergamaschi, 1997). The results indicate that no major partitioning between methanogenic pathways in the formation of CH_4 occurred during the growing season (Sugimoto and Wada, cited in Bergamaschi, 1997). Instead, only one pathway (acetate fermentation) was predominant throughout the season (region circled in red). Differences were found between slopes under the 20 - 28 °C temperature treatments and that at 32 °C, but could not be explained using the results in this research.

In contrary, no distinct relationship was found between $\delta^{13}\text{C}$ and δD values in pore water from planted tubs (Fig. 6.8), and the regression coefficients were very low ($r^2 = 0.017 - 0.268$), but positive.

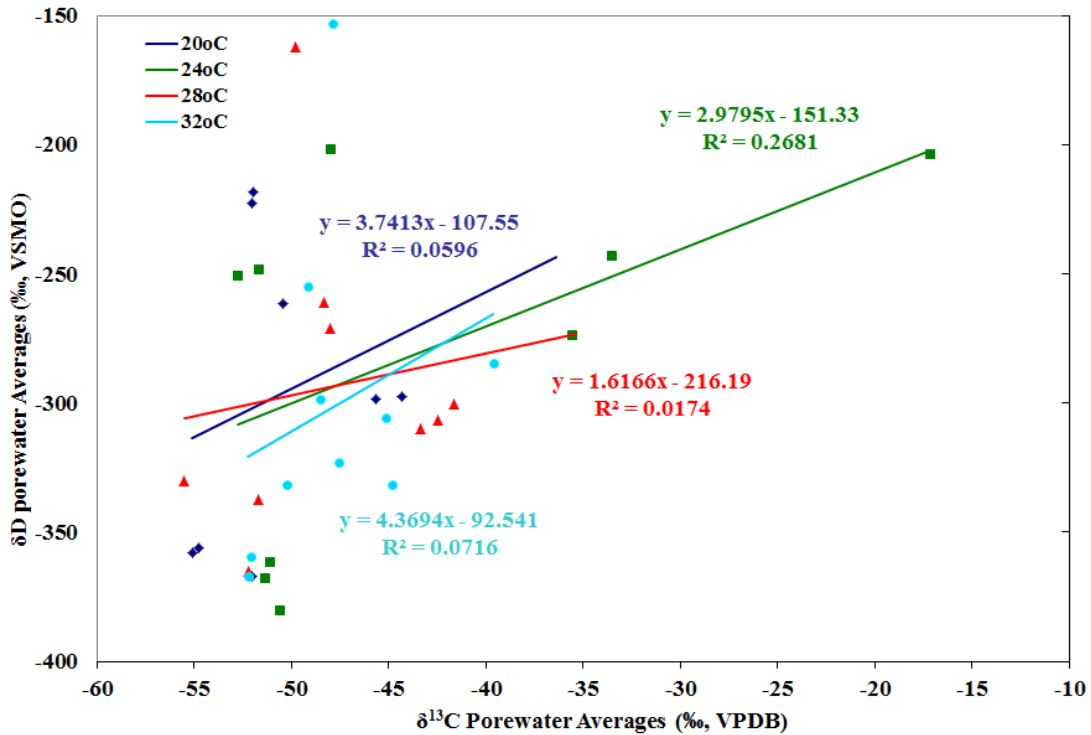


Fig. 6.8: Temperature effect on the relationship between isotopic composition, $\delta^{13}\text{C}$ and δD values in pore water from planted tubs.

Coleman et al. (1981) found positive linear relationships between the fraction of CH_4 oxidized and carbon and hydrogen isotopes, but with different regression coefficients. However, the fractions of CH_4 oxidized from $\delta^{13}\text{C}$ and δD were not similar in pore water. They concluded that the fractionations of the two isotopes decreased with temperature, and their responses were different under same temperature conditions.

These results were the first to pinpoint the importance of temperature in CH₄ fractionation.

6.4.4 Fraction of CH₄ Oxidized

The range of the fraction of CH₄ oxidized from $\delta^{13}\text{C}$ values was 46 to 87 % in planted tubs. In the controls, the percentage was low, ranging from 26 to 48 %. Higher percentage values found in planted tubs are consistent with the present knowledge of CH₄ oxidation in flooded rice soils. It is thought that plants, via roots provide aerobic conditions which promote CH₄ oxidation, since methanotrophs require oxygen to utilize CH₄ as their metabolic source of energy.

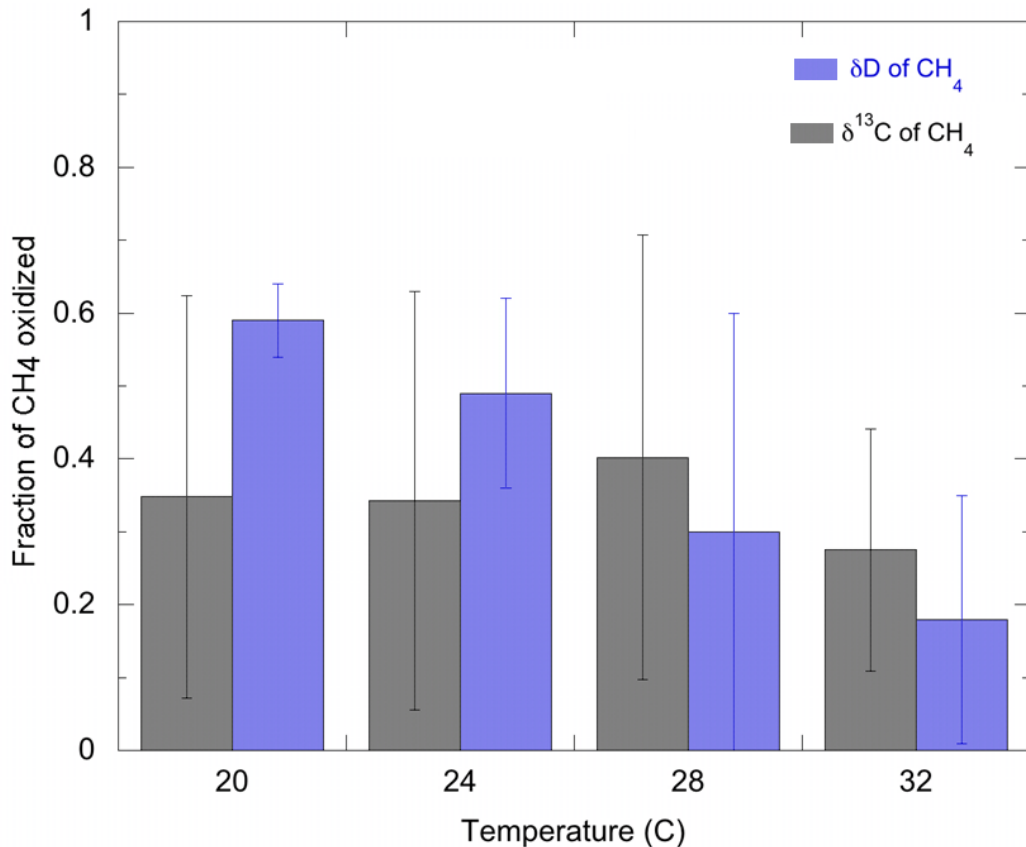


Fig. 6.9: Fraction of CH₄ oxidized as determined from δD and $\delta^{13}\text{C}$ values for planted tubs.

From δD values, the ranges in different temperature treatments were quite variable. The highest mean CH_4 oxidation fraction was 60 % (range: 55-65 %) occurred in the 20 °C tubs. Among different temperature treatments, the means generally decreased towards higher temperatures.

There were some discrepancies between the estimates of $\delta^{13}C$ and δD -calculated oxidation fractions. To date, there is little information in literature about the dual use of these isotopes as tracers in CH_4 oxidation experiments. However, the dual approach provides some means of understanding the uncertainty of these estimates. The reason for the variation in the two estimates could be a result of the differences in the temperature response of the α_{oxS} for $\delta^{13}C$ and δD . However, from $\delta^{13}C$ -calculated oxidation fractions, the optimum soil temperature for CH_4 oxidation was found to be 28 °C, consistent with values of between 25 °C and 30 °C reported in Chanton and Liptay (2000). The Q_{10} calculated from the fraction of CH_4 oxidized were 1-2 and 1.4-2.4 using $\delta^{13}C$ and δD values, respectively.

6.5 Conclusion

This research clearly showed that temperature is an important factor in CH_4 oxidation in rice ecosystems. However, further empirical observations are needed to refine methods used in determining the transport fractionation. The calculated Q_{10} values were less than average CH_4 fluxes and production. Such a scenario would promote increase in CH_4

production and emissions, resulting in a rising atmospheric CH₄ composition under global warming.

Chapter 7

Response of *mcrA* and *pmoA* Gene Copies and Methane Fluxes

to Soil Temperature Changes in Rice Microcosms

7.0 Summary

The feedback of global warming on methane emissions from rice agriculture was investigated by examining the link between populations of microbial consortia and elevated soil temperatures within the rhizosphere. Vertical soil profile samples were collected from temperature-controlled rice microcosms. The four water baths, set at different temperatures, each contained four tubs, with one control and three replicates. The soil samples were immediately frozen and stored at -80 °C, and were pulverized before DNA extraction. The concentration of the methyl coenzyme M reductase (*mcrA*) and particulate methane monooxygenase (*pmoA*) genes was determined by qPCR. All qPCR reactions were performed in double 25 µl reactions using the QuantiTect SYBR Green PCR kit. Quantitation of total *mcrA* gene copies was performed with primers described in Luton et al. (2002) at a final concentration of 0.6 µM (Wilson et al., 2010). An FID-equipped Gas Chromatography was used to measure the methane concentration in air samples collected from Plexiglas flux chambers as previously described. Our results showed that methanogens (*mcrA*) and methanotrophs (*pmoA*) were preferentially located to certain regions of the soil profile under different soil temperatures. *mcrA* copies were positively correlated to temperature, whereas no significant response of *pmoA* copies to temperature was observed. The implication of our results is that higher global temperatures will increase methanogen populations, but not as much for

methanotrophs, and hence increase CH₄ fluxes from rice agriculture. This result supports our work with isotopes described in the previous chapter.

7.1 Introduction

In rice fields, CH₄ is produced by three different pathways: carbon dioxide reduction, the methylotrophic pathway, and the acetate splitting pathway. The net CH₄ fluxes are controlled by the balance between microbial production and consumption of CH₄, and by physical processes such as diffusion, ebullition and ventilation. In anoxic freshwater environments, CH₄ production is accomplished by a complex microbial community consisting of hydrolytic, syntrophic, homoacetogenic, and methanogenic microorganisms that degrade organic matter under anaerobic conditions (Chin et al., 1999). Temperature is an important factor that controls the rate of CH₄ production, and methanogenesis pathway (Peng et al., 2008). In incubation experiments of rice field soils, studies have shown temperature has an effect on the structure and diversity of methanogenic Archaea. Chin et al. (1999) found that members of *Methanosarcinaceae* were predominant at 30°C, whereas at 15°C, the diversity of methanogenic Archaea was larger, comprising members of *Methanosaetaceae*. Conrad (1999) found that hydrogenotrophic and acetoclastic methanogenesis accounted for about 33 % and 67 % of total CH₄ production at 30 °C, respectively. The relative abundances in acetate-dependent *Methanosaetaceae* at low and high temperatures are believed to be regulated by the dependence of acetate on temperature (Peng et al., 2008, Chin et al., 1999). Acetate and H₂ or CO₂ are the predominant substrates of methanogenic archaea, with

the contribution from acetate being estimated at between 65 and 80% of CH₄ production (Chin et al., 1999). *Methanosaeta* and *Methanosarcina* are the only two genera of methanogens capable of breaking down acetate into methane and carbon dioxide .

Some of the CH₄ produced via methanogenesis is consumed and oxidized to CO₂ by aerobic methanotrophs (Hanson and Hanson, 1996; Ritchie et al., 1997; Roslev, 1997; Auman, 2000) and the fraction of CH₄ that is not oxidized by methanotrophs escapes from anaerobic environments to the atmosphere. Methanotrophs play a very significant role in maintaining the global CH₄ budget, and in moderating the impact of global warming on CH₄ production from anoxic soils. Methanotrophs are a unique group of methylotrophic bacteria (Ritchie et al., 1997) that utilize a variety of different one-carbon compounds such as methane, methanol, methylated amines, halomethanes, and methylated compounds containing sulfur as sole sources of carbon and energy (Hanson and Hanson, 1996). Two major phylogenetic groups of methanotrophs have been identified and are distinguished by their pathways for assimilation: Type I methanotrophs, which are *Gammaproteobacteria*, and Type II methanotrophs, which are *Alphaproteobacteria* (Hanson and Hanson, 1996). Type I methanotrophs assimilate formaldehyde, produced from the oxidation of methane (via methanol) using the ribulose monophosphate pathway. Type II methanotrophs use the serine pathway for formaldehyde assimilation. The pathway conversion efficiencies of Type I and Type II methanotrophs are estimated to be about 65 to 80 % and 40 to 60 %, respectively (Auman, 2000).

Given the complex response of both methanogen and methanotroph communities to various environmental factors, and the IPCC temperature projections of between +1.8 °C (B1 “optimistic” scenario) and +4 °C (A1FI “business as usual” scenario) by 2100 (IPCC, 2007c), it is important to understand how increasing temperatures may affect the balance between CH₄ production and consumption, and hence, the change in CH₄ emissions. In this work, we used the methyl coenzyme M reductase (*mcrA*) and particulate methane monooxygenase (*pmoA*) genes as proxies for methanogen and methanotroph populations within the rice rhizosphere, respectively. The *mcrA* gene codes for a subunit of the protein complex that catalyzes the final step of methanogenesis in all methanogenic Archaea by reducing coenzyme M binding methyl group to methane (Watanabe et al., 2009), while the *pmoA* is the α -subunit of the particulate methane monooxygenase (Kolb et al., 2003). We also present a quantitative analysis of the relationships between *mcrA* and *pmoA* abundance ratios at different soil horizons and soil temperatures and CH₄ fluxes.

7.2 Aims and Objectives

In this chapter, methods used to detect and quantify methanogens and methanotrophs are presented. Objectively, the experiments were aimed at: (I) Quantifying changes in their populations in response to changes in temperature over the growing season; (II) Determine the location of methanogens and methylootrophs within the depth profile of the root zone; (III) and to investigate the possible link between the diurnal variation in fluxes and bacterial activity, and their response to changes in soil temperature.

7.3 Materials and Methods

Four different temperature treatments, each comprising one unplanted (control) tub and three tubs planted with *Oryza sativa* L. 'M-103' (- a medium-grain, early maturing semi-dwarf rice cultivar), were heated using a water bath system whose temperature was maintained as described in Chapter 2.

7.3.1 Preparation and Collection of Soil Samples

Soil samples were collected every two weeks from rice tubs using 3/4" x 12" Butyrate Plastic Liners (Forestry Supplies Inc., USA). Because these liners could not be autoclaved, they were first cleaned thoroughly by soaking them in Alconox for about 24 hours and washed three times using de-ionized water, rinsed with 95 % ethanol, and wrapped in aluminum foil before they were put in a 60 °C drying oven for 12 hours. With sterile gloves, these corers were manually driven into the rice tubs to extract the soil vertical profile sample within the root zone. The duplicate plastic liner-filled samples drawn from each set-up were cut into four equal columns, put into sterile Whirl-packs (VWR International, LLC, USA), and immediately frozen at -80 °C.

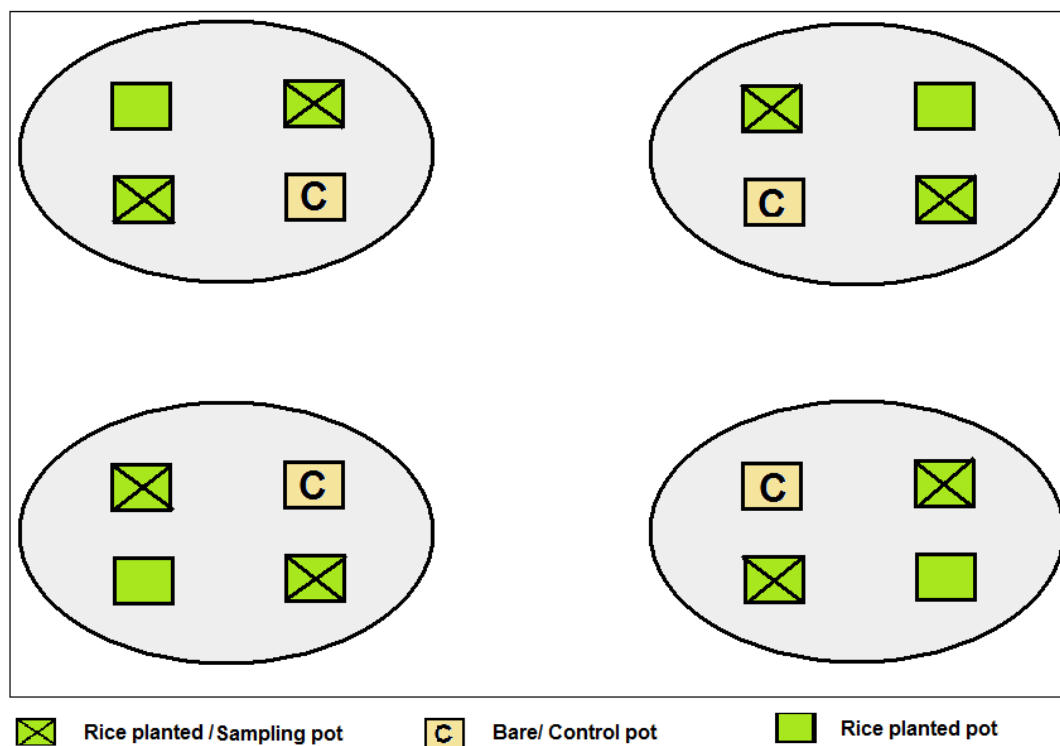


Fig.7.1: Schematic of the replicates (Rice planted tubs) and tubs from which the soil samples for population studies will be taken (rice-planted sampling tubs), and controls for each set of experiment (unplanted/Control tubs).

7.3.2 Soil Microbial DNA Extraction, Quantitation and Purity

Prior to DNA extraction, individual soil samples were homogenized using sterile mortars and pestles. DNA was extracted from approximately 1.4 g (wet weight) of soil using the UltraClean™ Soil DNA Kit (Mo Bio Labs, Inc., USA) following the manufacturer's instructions. The DNA yield was determined using a UV/Vis NanoDrop ND-1000-V3.7 Spectrophotometer (Thermo Fisher Scientific Inc., USA). DNA absorbance was measured at 230 nm, 260 nm and 280 nm. The absorbance ratios at 230/260 nm and 260/280 nm were calculated by the NanoDrop ND-1000 software.

7.3.3 qPCR Detection of Methanogens and Methanotrophs

All qPCR reactions were performed in duplicate 25 µl reactions using the QuantiTect SYBR Green PCR kit (Qiagen, Valencia, California) run on MJ Mini Opticon Real-Time PCR System (BioRad, Hercules, California). Melting curves were performed at the end of each reaction to ensure product integrity. Quantitation of total *mcrA* gene copies was performed with *mcrAf* and *mcrAr* primers (Luton et al., 2002) at a final concentration of 0.6 µM, following the method of Wilson et al., (2010). The thermal protocol was as follows: 95 °C for 15 min, 40 cycles of 94 °C for 30s, 56 °C for 30 s, 72 °C for 1 min, followed by a single cycle of 82 °C for 10 s. Plasmids containing the *mcrA* gene were used as quantitation standards as previously described (Wilson et al., 2010). In a similar fashion, quantitation of *pmoA* genes was carried using primers A189f (Holmes et al., 1995) and mb661r (Costello and Lidstrom, 1999). The thermal protocol was as follows: 95 °C for 15 min, 45 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min, followed by a single cycle of 84 °C for 10 s. The protocol (reaction) temperatures and cycling for *mcrA* analysis were as shown in Fig. 7.2.

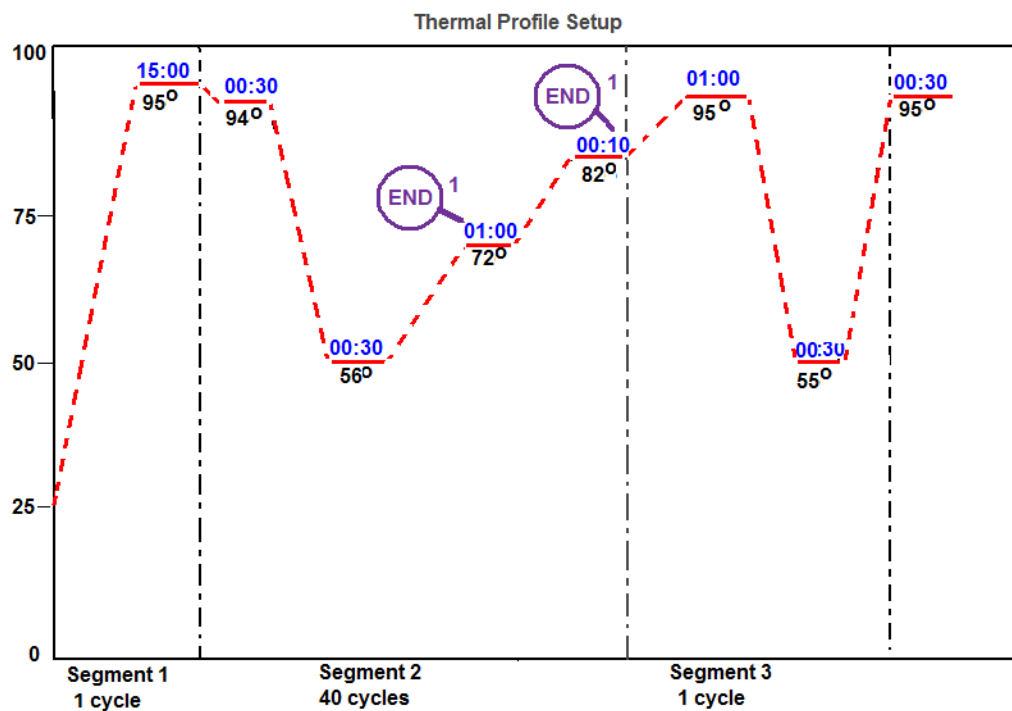


Fig. 7.2: Protocol for the qPCR thermal profile set-up for *mcrA* gene quantitation were as follows: 95 °C for 15 min, 40 cycles of 94 °C for 30s, 56 °C for 30s, 72 °C for 1 min, followed by a single cycle of 82 °C for 10s.

Genomic DNA of *Methylococcus capsulatus str. Bath* (ATCC 33009D) was used to generate standard curves for quantitation. qPCR assays of the *mcrA* and *pmoA* genes with linear calibration standard curves with regression coefficients >0.990 were considered. Both *mcrA* and *pmoA* gene concentrations were normalized to dry weight of soil.

7.3 Statistical Analysis

One-way analysis of covariance (ANACOVA) was used to test the null hypothesis (H_0) that there was no difference between the coefficients of regressions between *pmoA* and

mcrA copies, based on a 5 % level of significance. A t-statistic was computed as the difference between the two slopes divided by the standard error of the difference between the slopes, with (N_1+N_2-4) degrees of freedom, where N_1 and N_2 were the number of points on each regression equation. H_0 was rejected for any $p < 0.05$.

7.5 Results and Discussion

7.5.1 DNA Purity and Yield.

To check if the extracted DNA samples contained any qPCR amplifiable quantities, absorbance ratios at 260nm/230nm (DNA/humic acids) and 260 nm/280 nm (DNA/protein) were measured on a UV/Vis NanoDrop ND-1000-V3.7 Spectrophotometer. Medians of the absorbance ratios at 260 nm/230 nm and 260 nm/280 nm ranged from 0.4-0.6 and 1.75-1.95, respectively.

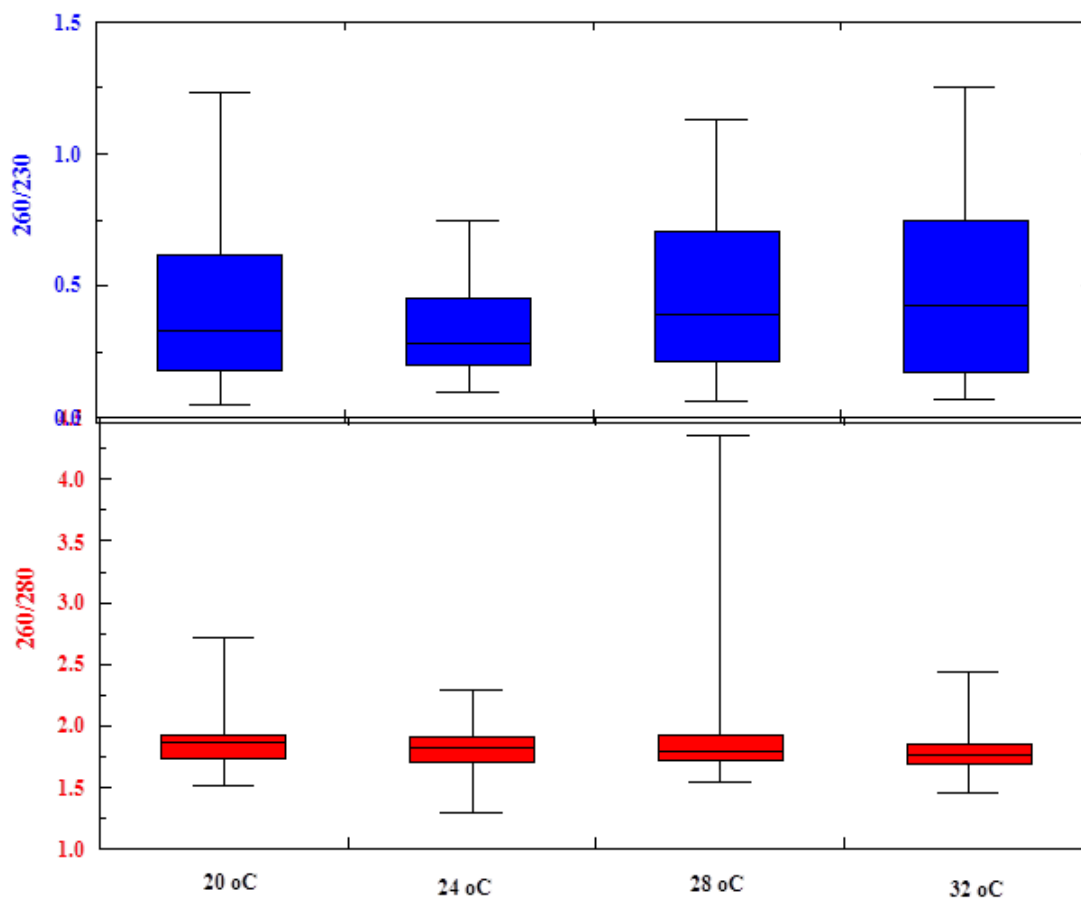


Fig. 7.3: Ratios of 260/230 (e.g. >2) and 260/280 (e.g. >1.7) are indicative of pure DNA, while the low ratios are indicative of humic acid and protein contamination, respectively.

The results indicated that DNA was contaminated with more humic acid-like compounds than proteins, and that the level of humic acids in the DNA increased during the course of the experiment (Fig. 7.3).

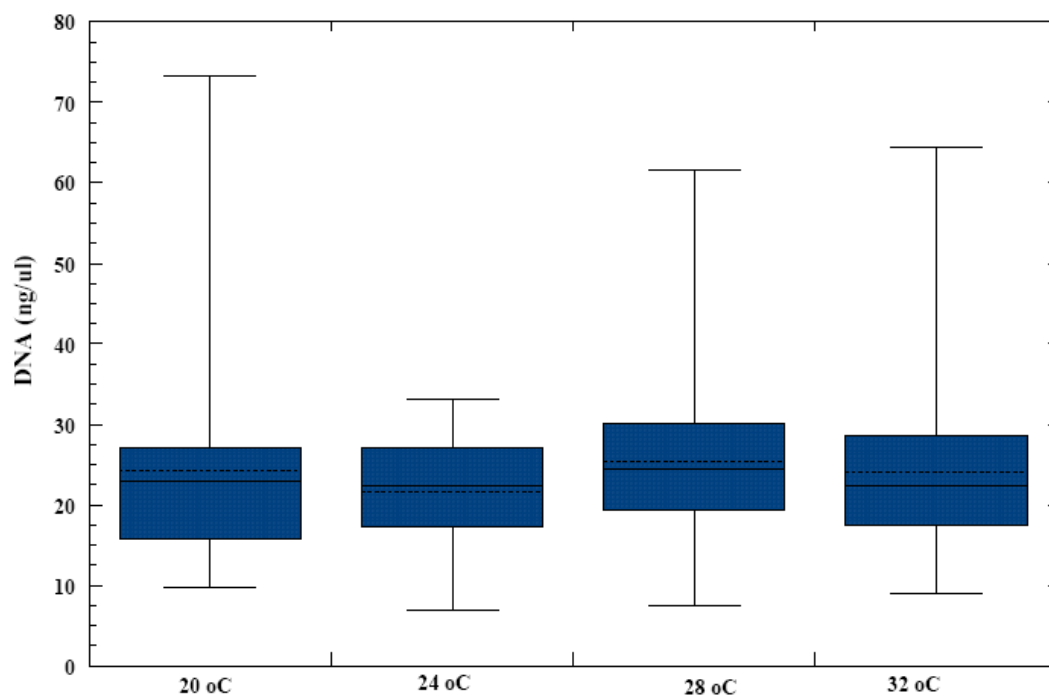


Fig. 7.4: Total DNA yields ranged from of 10 to 45 μg of DNA per μl . Data showed no notable differences across different temperature treatments.

The level of humic acid contamination slightly increased during the season. The low ratio at 260 nm/230 nm was an indication that proteins, possibly from bugs in the rice stalks, were the major contaminants of our DNA assays. The total yield ranged from 10 to 45 $\text{ng } \mu\text{L}^{-1} \text{g}^{-1}$ (dry soil), with highest values in 5-15 cm soil depth (Fig. 7.5).

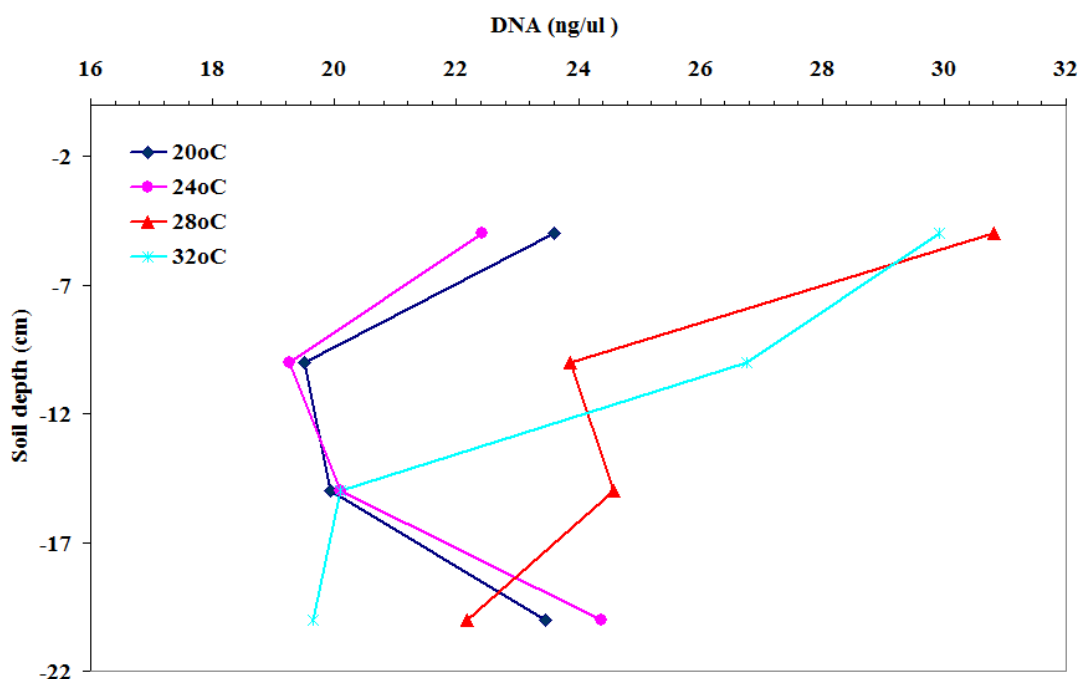


Fig. 7.5: Average values of DNA concentration at different soil horizons at different soil temperature treatment.

Generally, no notable significant change was observed between set-ups at different temperatures, with the mean remaining almost constant at $22 \pm 4 \mu\text{L}^{-1}\text{g}^{-1}$ (dry soil).

7.5.2 Abundance of *mcrA* Genes

The detection limit of 10^2 of *mcrA* copies per assay was achieved. The *mcrA* gene abundance ranged from 5×10^5 to 3×10^6 copies g^{-1} of dry soil (Fig. 7.6). The same order of magnitude has been reported in literature (Bodelier and Frenzel, 1999; Bosse and Frenzel, 1997; Gilbert and Frenzel, 1995; Joulian et al., 1997). Orders of magnitude as low as 10^4 copies g^{-1} (dry soil) were also reported in soils from Japanese rice fields

(Eusufzai et al., 2010), while higher values of the order of between 10^5 and 10^7 copies g^{-1} (dry soil) were also found in soils from 15 paddy fields (five different soil types) in north-east China (Wang et al., 2010).

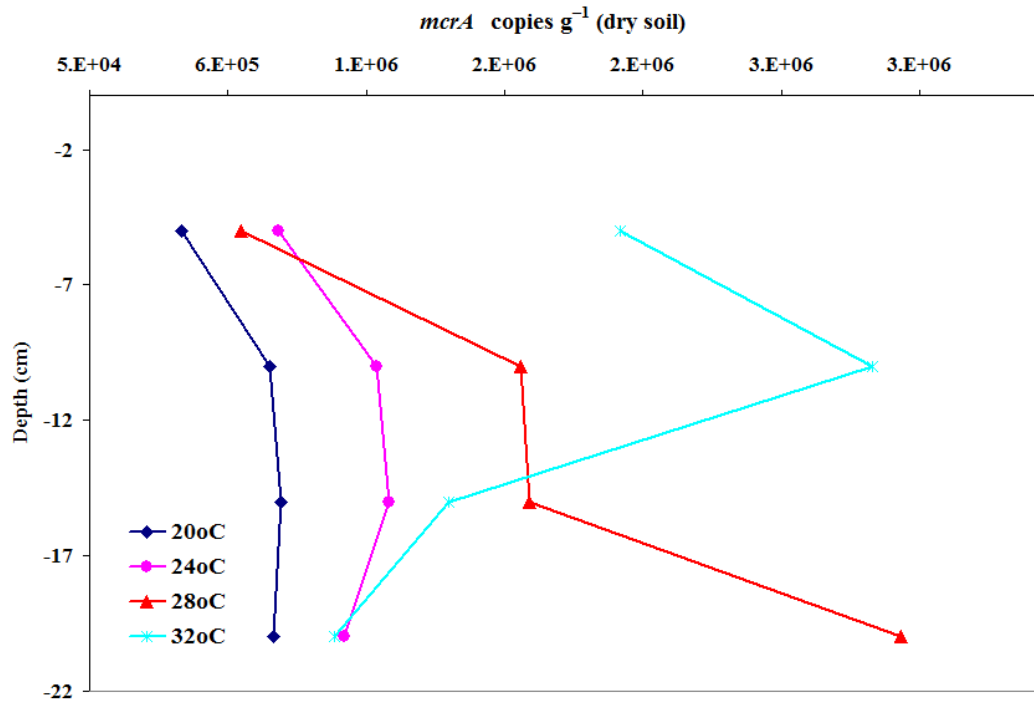


Fig. 7.6: Average values of *mcrA* copies at different soil horizons at different soil temperature treatment. Highest *mcrA* copies were found at 28 and 32 °C.

Considering the variation in the orders of magnitude of *mcrA* copies from incubation and field studies of soils from rice ecosystems, results in this study fell within the median range, and may be considered to be an estimate of the average abundance of *mcrA* copies in most rice paddies.

A temperature increase from 20 to 32 °C resulted in a five-fold increase in *mcrA* copies in the 5-10 and 10-15 cm soil zone. Similar results have been observed in root and straw amended rice soil core incubation studies (Peng et al., 2008). Within this zone, the *mcrA* copies were low at the start of the season, approaching an almost steady-state between 54 and 146 days after transplanting (DAT). The results are consistent with observations from Chin et al. (1999) in which a similar trend was observed from rice fields.

7.5.3 Abundance of *pmoA* Genes

The abundance of *pmoA* genes ranged from 5×10^4 to 4×10^5 copies g^{-1} of dry soil (Fig. 7.7). In general, our results were comparable to those found in other flooded rice field soils in which orders of magnitude were 10^4 - 10^6 copies g^{-1} of dry soil (Kolb et al., 2003; Bosse and Frenzel, 1997).

Values of methanotroph populations of orders of magnitude as high as 10^7 copies/g (dry soil) have also been found in the top 0-2cm soil cores from straw and straw-burned rice paddies under desaturated soil conditions and early period following flooding/planting (Macalady et al., 2002). Macalady et al. (2002) found that methanotroph growth was highest close to the soil-water interface (0-2cm soil layer) but lower in bulk soil without rice roots ($<10^6$ copies/g dry soil) in the zones below the top layer.

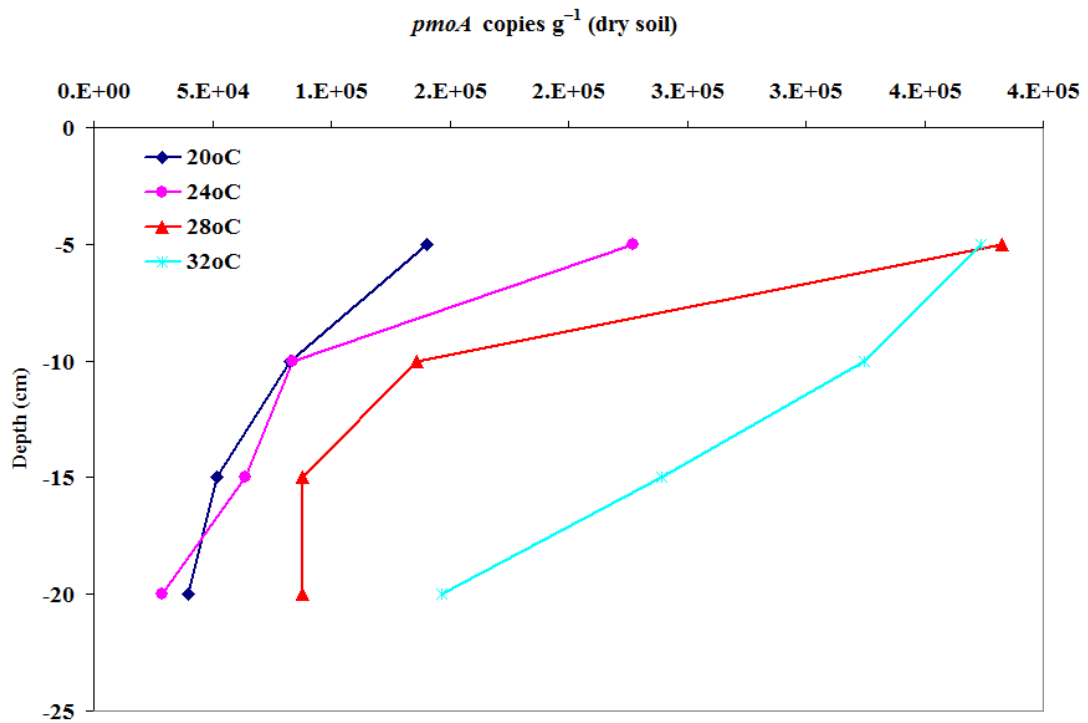


Fig. 7.7: Average values of *pmoA* copies at different soil horizons at different soil temperature treatment. Highest *pmoA* copies were found at 28 and 32 °C.

The differences could be a result of the differences in the soil carbon content and texture. Another explanation for these differences is possibly the effect of differences in gas conductance (O₂ supply into the rhizosphere) in different rice cultivars. A higher number of *pmoA* copies was found in the aerobic top soil horizon (0-5 cm), an indication that methanotrophy is an aerobic process (Fig. 7.8).

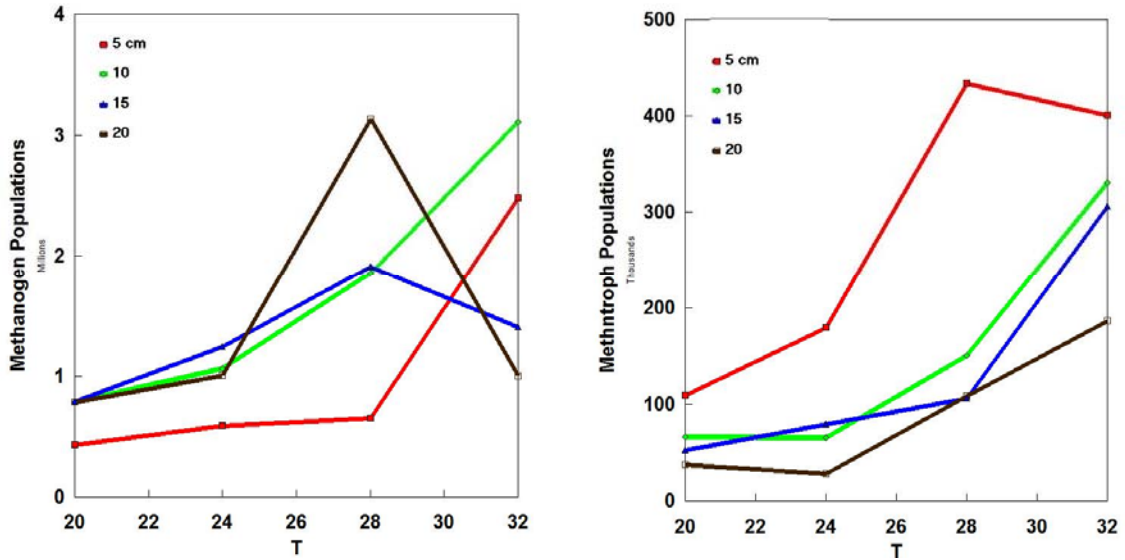


Fig. 7.8: Methanogen and methanotroph populations at different soil horizons at different soil temperature treatments.

Across different temperature treatments, the highest number of *pmoA* copies occurred at highest temperature (Fig. 7.9). Across different temperatures, no significant change in *pmoA* abundance was observed at other soil horizons, except at 32°C. This observation may reflect the difference in the response of different methanotrophic bacteria to elevated temperatures. For example, a change in the methanotrophic community composition in rice field soil at different temperatures has been reported (Chin et al., 1999; Mohanty et al., 2007; Peng et al., 2008).

7.5.4 Relative Abundance between *mcrA* and *pmoA* Genes

The relatively low number of *pmoA* to *mcrA* copies suggested that CH₄ oxidation rate was generally lower than production rate given the high CH₄ pore water concentration levels that were found in our experiments. Because of fast transport, oxidation in paddy

fields can be as low as around 30 % of production (Bodelier et al., 2000). If this percentage roughly translates to the relative numbers of *pmoA* and *mcrA* copies in rice fields, then results in this research in which the oxidation rates were between 20 and 60 % (in Chapter 6) are consistent with those from other studies. In addition, increasing the temperature, increases organic degradation and increases substrate for CH₄ production and in the process, depletes O₂ and creates anaerobic conditions. The other reason for the low number of *pmoA* copies is that the soils were amended with chemical fertilizers, which have been found to inhibit CH₄ oxidation and decrease carbon conversion efficiency (Roslev et al., 1996).

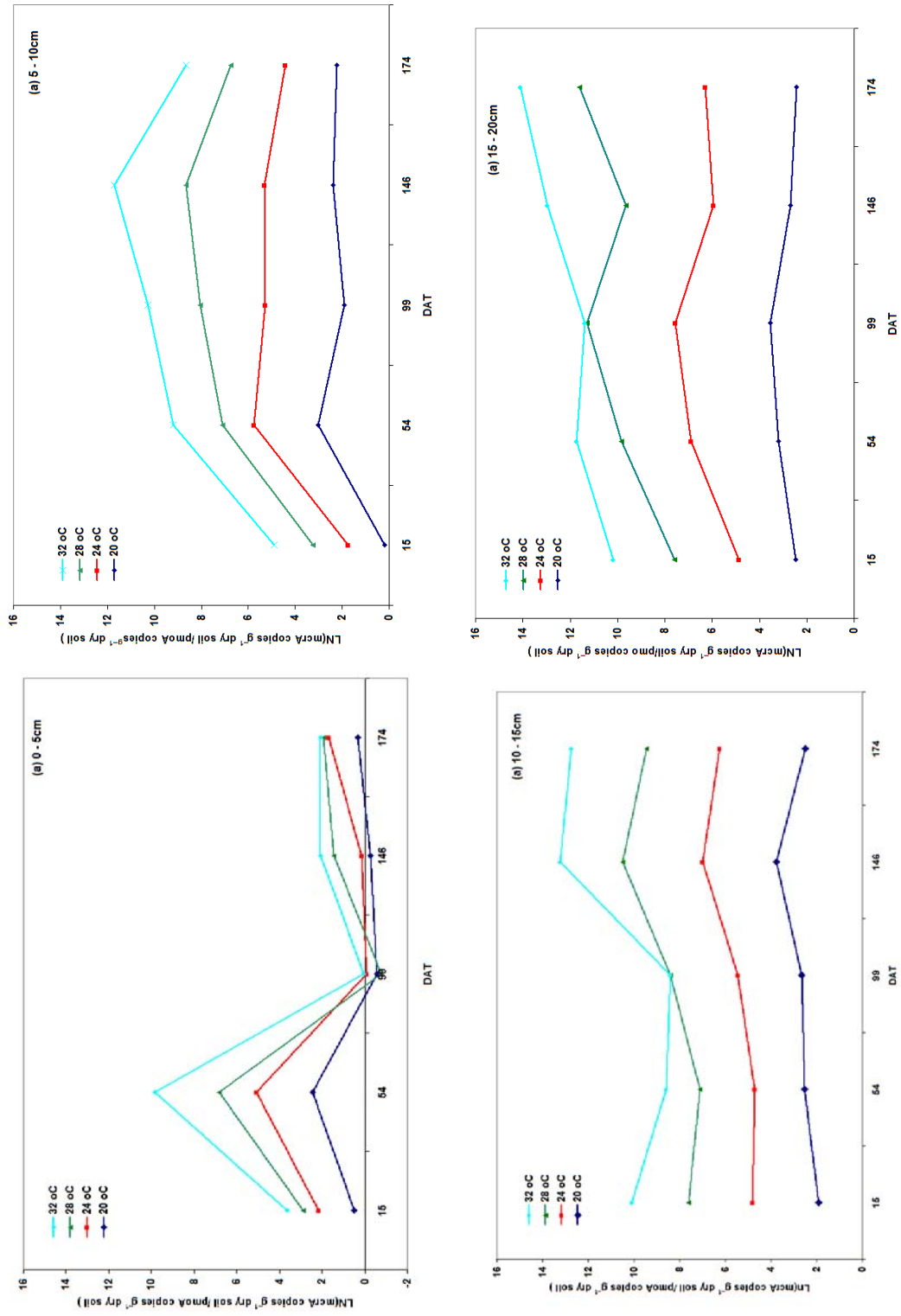


Fig. 7.9: Relative abundance of *mcrA* and *pmoA* (copies g⁻¹ dry soil) at different depths

For all temperature treatments, the lowest number of *mcrA* copies was located in the top soil layer (0-5 cm), while *pmoA* copies were mostly abundant in the 0-5 cm oxygenated soil horizon in all set-ups, but remained fairly constant in the 5-10, 10-15 and 15-20 cm horizons. Ratios between *pmoA* and *mcrA* copies were consistently higher at 28 °C than at other temperatures (Fig. 7.9). In particular, the highest ratios occurred in the 5-10, 10-15 and 15-20 cm soil cores. Within the CH₄ production zone (5-10 and 10-15 cm), well-defined stratification of *mcrA* to *pmoA* ratios was observed. This, according to our knowledge, is the first time these results have been obtained.

7.5.5 Regression between *pmoA*, *mcrA* Copies and Temperature

To gain insight into the dependence of *pmoA* on *mcrA* copies and indirectly measure oxidation and methanogenesis potential and temperature, linear regression curves were plotted for different temperatures and horizons (Fig. 7.10). Regression coefficients between *pmoA* and *mcrA* copies ranged from 0.00-0.92, with highly significant values in the 5-10 and 10-15 cm soil horizons, and almost zero elsewhere. To check the dependence of methanotrophs on the population of methanogens, regression coefficients between the two were plotted against soil depth (graphs not shown), and the highest values were found around 5-10 and 10-15 cm horizons, an indication that methanotroph populations are governed by the availability of substrates from methanogens. Thus, the greater the growth of methanogen population, the more CH₄ is produced, which in turn stimulates the growth of methanotrophs which depend on the CH₄ from methanogenesis as their substrates.

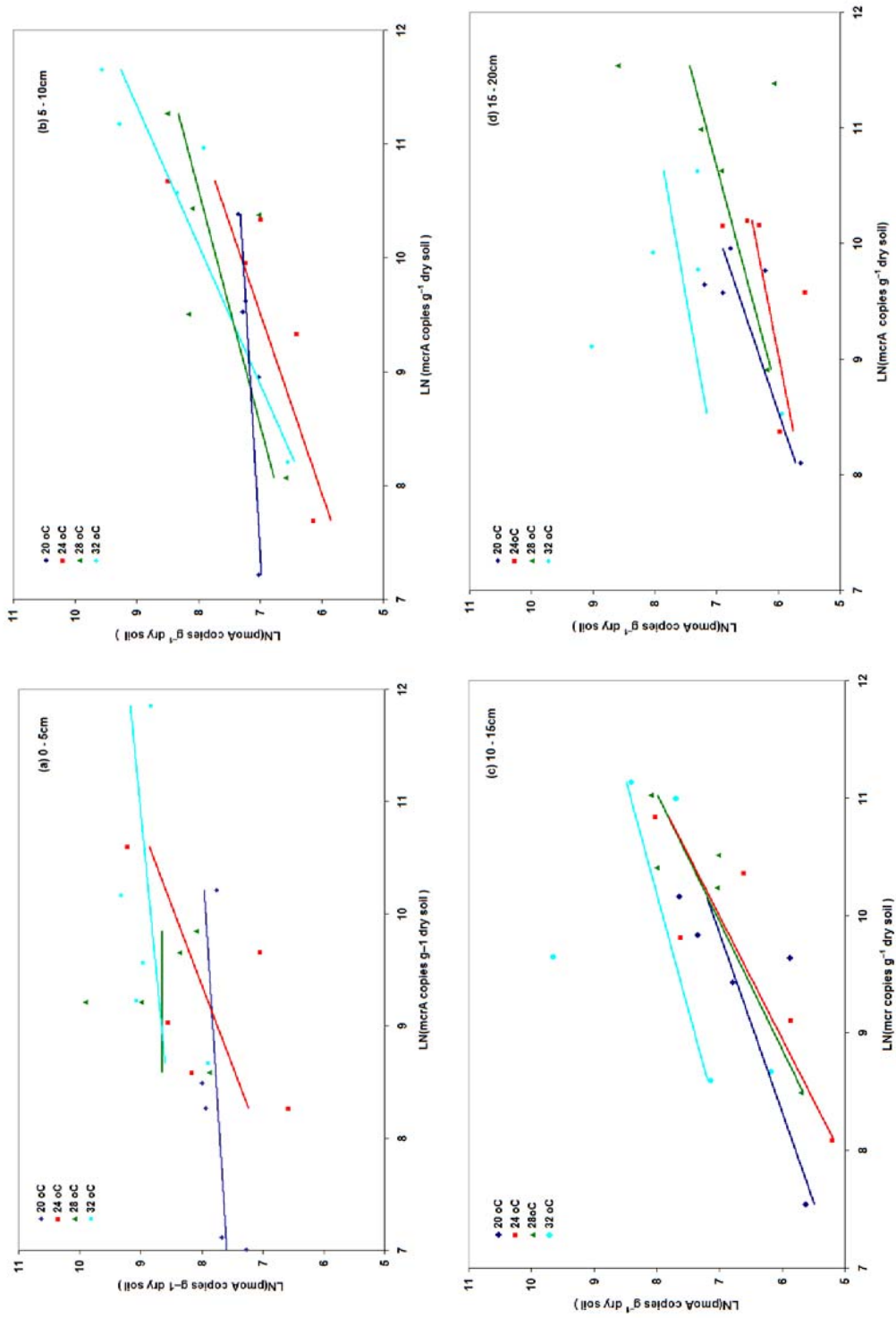


Fig. 7.10: Regressions: natural log (*pmoA* copies g⁻¹ dry soil) versus natural log (*mcrA* copies g⁻¹ dry soil)

Freitag et al. (2010) found regression coefficients between 0.51 and 0.91 from only two soil layers of 0-10 and 10-20 cm from a blanket peat bog. While the observed trends reported by Freitag et al. (2010) are similar to our findings, they found consistently high values in the homogeneous samples from only two soil layers. Homogenization of the samples in the two horizons may have concealed more information about the actual profile of the regression coefficients within the 0-20cm soil depth.

Consistent with our results, Joulain et al. (1997) found methanotrophs to be positively correlated with methanogens within the main CH₄ production zone. Their results showed that the correlation was strong for *acetotrophs* and *methylotrophs* and weak for *hydrogenotrophs* and concluded that the second group depended on substrates produced by the first group. In our results (Table 7.1), the high regression coefficients in the main CH₄ production zone show that methanogens control methanotroph substrate availability, and hence, partly determine the abundance of methanotrophs.

A statistical test (5 % level of significance) of the difference between the regression coefficients (Tab. 7.1) using ANACOVA indicated that the slopes between *pmoA* and *mcrA* copies were not significantly different from each other in the 0-5 cm and 15-20 cm. Across different temperature treatments, the null hypothesis was rejected within the 5-10 cm soil horizon, meaning, that the regression coefficients were statistically different ($p < 0.05$) for different temperatures and those in other soil layers.

Table 7.1. Regression of *pmoA* (copies g⁻¹ dry soil) to *mcrA* (copies g⁻¹ dry soil).

Temp. °C	Regression			
	0-5 cm depth	5-10 cm depth	10-15 cm depth	15-20 cm depth
20	y = 0.1142x + 6.7884 r ² = 0.2645	y = 0.1082x + 6.2003 r ² = 0.7159	y = 0.657x + 0.5344 r ² = 0.5847	y = 0.6338x + 0.5795 r ² = 0.5787
24	y = 0.6984x + 1.462 r ² = 0.3573	y = 0.6298x + 1.0053 r ² = 0.6544	y = 0.9524x - 2.5185 r ² = 0.7647	y = 0.3679x + 2.6794 r ² = 0.3165
28	y = 0.0005x + 8.6413 r ² = 8E-08	y = 0.484x + 2.8714 r ² = 0.5097	y = 0.9142x - 2.096 r ² = 0.8268	y = 0.5019x + 1.6428 r ² = 0.2716
32	y = 0.1803x + 7.0367 r ² = 0.1623	y = 0.8161x - 0.257 r ² = 0.8447	y = 0.5079x + 2.8262 r ² = 0.2229	y = 0.3336x + 4.314 r ² = 0.056

7.5.6 Relationships between CH₄ Fluxes and *mcrA* and *pmoA* Copies

CH₄ fluxes increased with increasing abundance of *mcrA* copies (Fig. 7.11). The observed trends between CH₄ fluxes and *mcrA* copies were strikingly similar to those observed between CH₄ fluxes and *mcrA* gene transcript abundances reported in literature (Freitag et al., 2010; Freitag and Prosser, 2009).

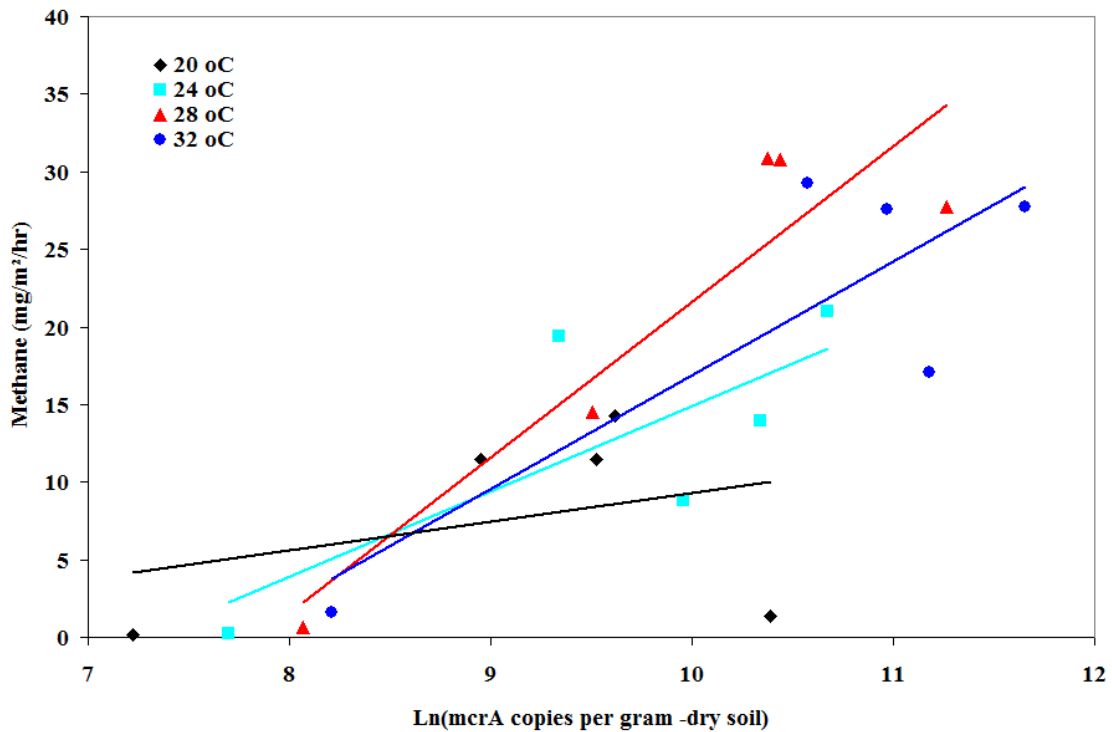


Fig. 7.11: CH₄ fluxes vs *mcrA* a copies at 5-10 cm depth

From soil sample results, the highest regression coefficient was found at 28 °C ($R^2 = 0.850$), consistent with results from incubation experiments of temperate peat soil slurries by Freitag and Prosser (2009), in which the highest CH₄ production rates and mean mRNA transcript/gene abundance ratios occurred between 25 and 30 °C ($R^2 = 0.790$).

No well-defined relationship was observed between *pmoA* copies at any level and CH₄ fluxes and soil temperature (Fig. 7.12). Both regression coefficients and slopes were generally low indicating that there is no direct link between methanotroph population and CH₄ fluxes. Only in one particular case (0-5 cm, 20 °C) was the regression

coefficient significant. In other studies, (Freitag et al., 2010), regression coefficients ranged between 0.21-0.45 (n = 4-5).

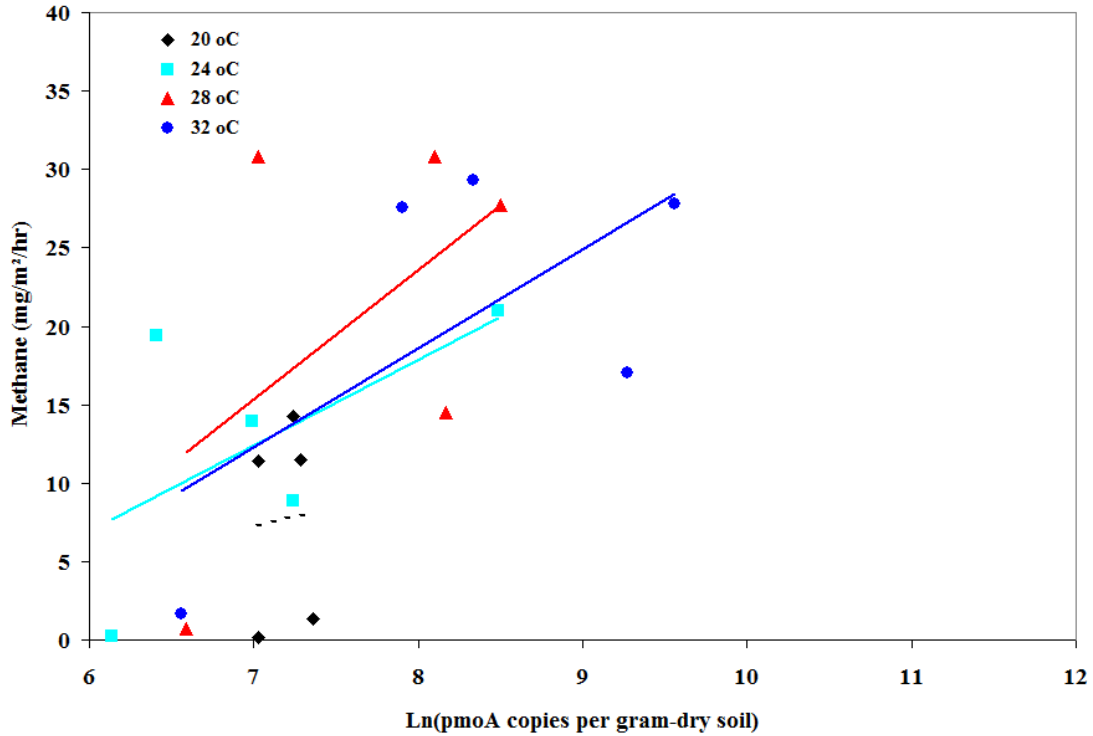


Fig. 7.12: CH₄ fluxes vs *pmoA* copies at 5-10 cm depth

7.5.7 Q₁₀ of Methanogens and Methanotrophs

In general the population of methanogens was found to be higher but less responsive to soil temperature change than methanotrophs (Fig. 7.13). The Q₁₀ of methanogens and methanotrophs were calculated based on the populations:

$$Q_{10} = \left[\frac{P(T_o + \Delta)}{P(T_o)} \right]^{10/\Delta}$$

Where P(T₀) is the population at the base temperature, T = 20 °C and P(T + Δ) is the population at any temperature.

The Q_{10} of methanogens generally decreased from the top soil horizon to the bottom, while those for methanotrophs slightly increased with soil depth.

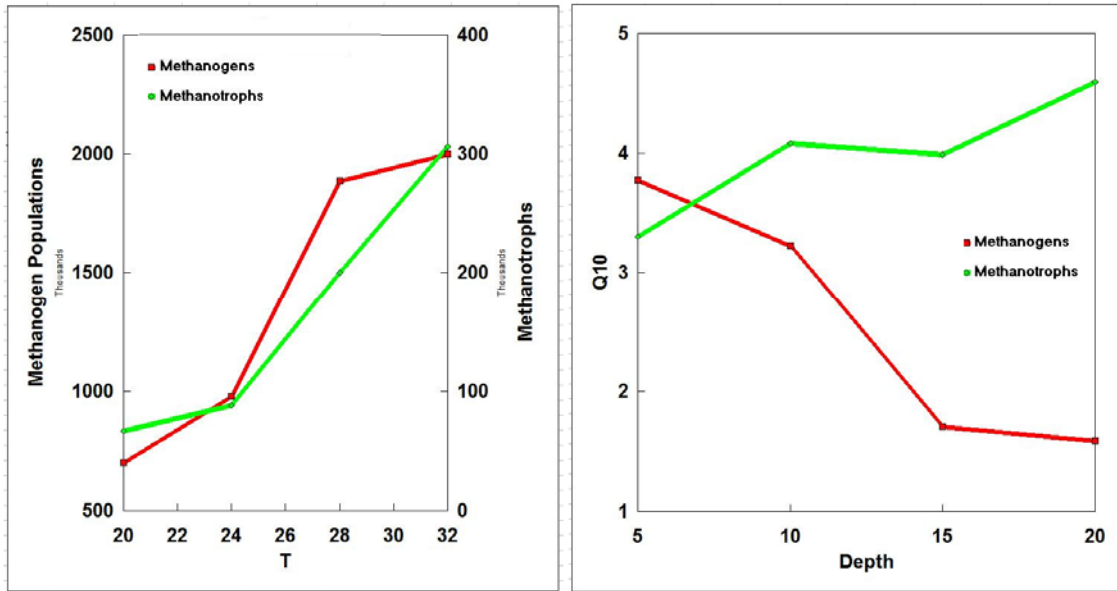


Fig. 7.13: (a) Dependence of methanogen and methanotroph populations on soil temperature. (b) Q_{10} of methanogen and methanotrophs for different soil horizons.

The calculated Q_{10} s values of methanogens were respectively 1.5 and 4.6 for the lower and upper limit, with a seasonal average of 2.6 (90 % confidence level). For methanotrophs, the Q_{10} s were between 2.3 and 6.4, with a seasonal average of 3.8. Within the 10 -15 cm, the values were closer to each other.

During the season, the Q_{10} s were generally higher during mid-season, and lower elsewhere. The highest change in values for Q_{10} values of methanogens were recorded in the top soil horizon (0 - 5 cm), while for methanotrophs the change occurred in the 15 - 20 cm soil layer (7.14).

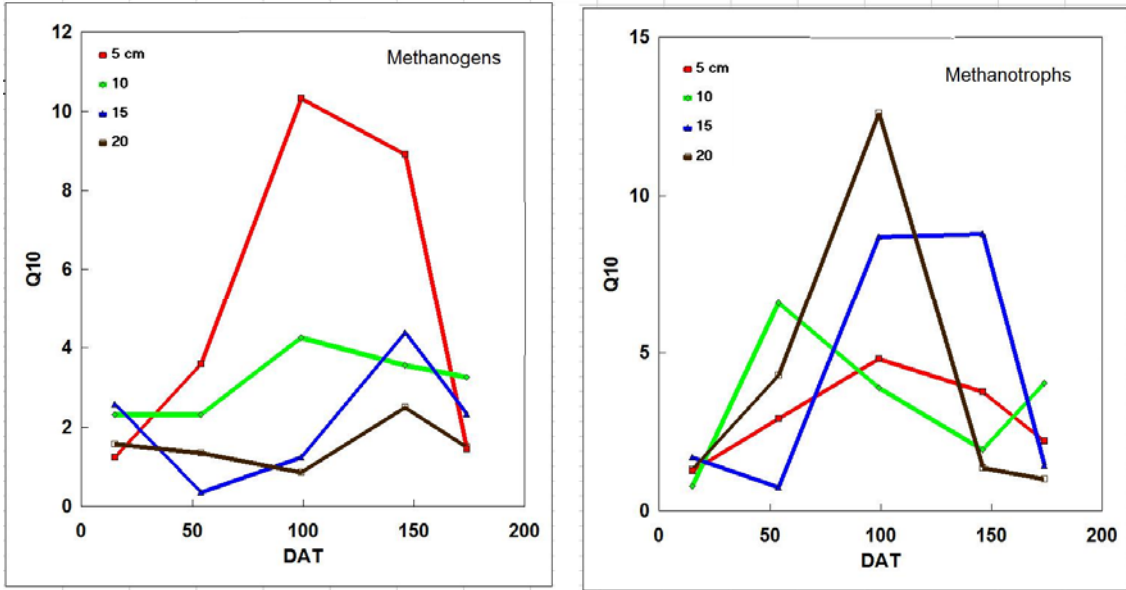


Fig. 7.14: Variation of Q_{10} values of methanogens and methanotrophs during the season for different temperature treatments and soil depth.

7.6 Conclusion

This study showed no significant response of *pmoA* to temperature, while *mcrA* populations were positively correlated to temperature. Below the 0-5 cm horizon, the *mcrA* copies were more abundant than *pmoA*. High populations of *mcrA* were weakly correlated to *pmoA* population in the 5-15 cm horizon, showing that the relationship between these populations may be more complex than just a linear approximation. The general increase in CH_4 fluxes with elevated temperature could stimulate heterotrophs that deplete O_2 and create better conditions for increased CH_4 production, but are not conducive for *pmoA*. If these results constitute a representative sample of the response of methanogens and methanotrophs to temperature in all rice field soils, then global

warming is expected to stimulate CH₄ fluxes, and hence increase the global atmospheric CH₄ budget from rice agriculture and possibly wetlands.

Chapter 8

Nitrous Oxide Emissions from Rice Agriculture under Elevated Soil Temperatures

8.0 Summary

One of the unknowns in predicting the future concentrations of N₂O is how the biogenic emissions may change with increasing temperatures. Here results of N₂O emissions from soil warming experiments to determine the Q₁₀ of fluxes and production from rice ecosystems under different soil temperature treatments are reported. Experiments were conducted between spring of 2009 and summer of 2010. Fluxes were measured using static Plexiglas flux chamber in the same manner as for methane. The variations and comparisons of pore water N₂O concentrations at four different soil depths are also reported here. N₂O fluxes were characterized by high temporal variations, ranging from values as high as 1800 µg-N/m²/hr during the first two weeks following flooding to almost zero and occasionally negative thereafter. Weak correlations were found between N₂O fluxes and temperature, with Q₁₀ values ranging from 0.5 to 3.3. High production rates coincided with periods of fertilization, with highest rate occurring at the onset of the growing season. The Q₁₀ values of production varied between 0.4 and 3. These results indicate the existence of a positive feedback between soil temperature and N₂O emissions from paddy soils. The implication of these results will be an increase in N₂O emissions from rice paddies under global warming. However, on a global scale, in addition to the direct effect of soil temperature, the pattern and distribution of future N₂O emissions from rice paddies (and possibly similar ecosystems) will also be

determined by how much global warming will affect the pattern and distribution of water tables, since the dominant processes leading to N₂O production also depend on soil moisture content.

8.1 Introduction

Of the estimated 20 Tg-N₂O annual global N₂O emissions (Smith, 1997; Smith et al., 1998), about 2 - 3 Tg/yr originates from cultivated soils. Emission rates in agricultural systems are related to the quantities of N used as fertilizers and, where relevant, to recent land use change (Smith, 1997). The constantly increasing atmospheric concentration, as well as the disparities in reports on the effect of temperature on N₂O emissions from rice agriculture underscores the urgent need to better understand the likely feedback between global warming and these emissions. Per molecule basis, N₂O is 310 times more potent as a greenhouse gas than CO₂. Because of its contribution to global warming, and participation in stratospheric ozone destruction, N₂O has been studied extensively over the past three decades. Application of N-fertilizer has been identified as a major source of N₂O in agricultural soils (Towprayoon et al. 2005; Yang et al., 2003; Stehfest and Bouwman, 2006), contributing about 58 % of total anthropogenic emissions (Smith et al., 2007).

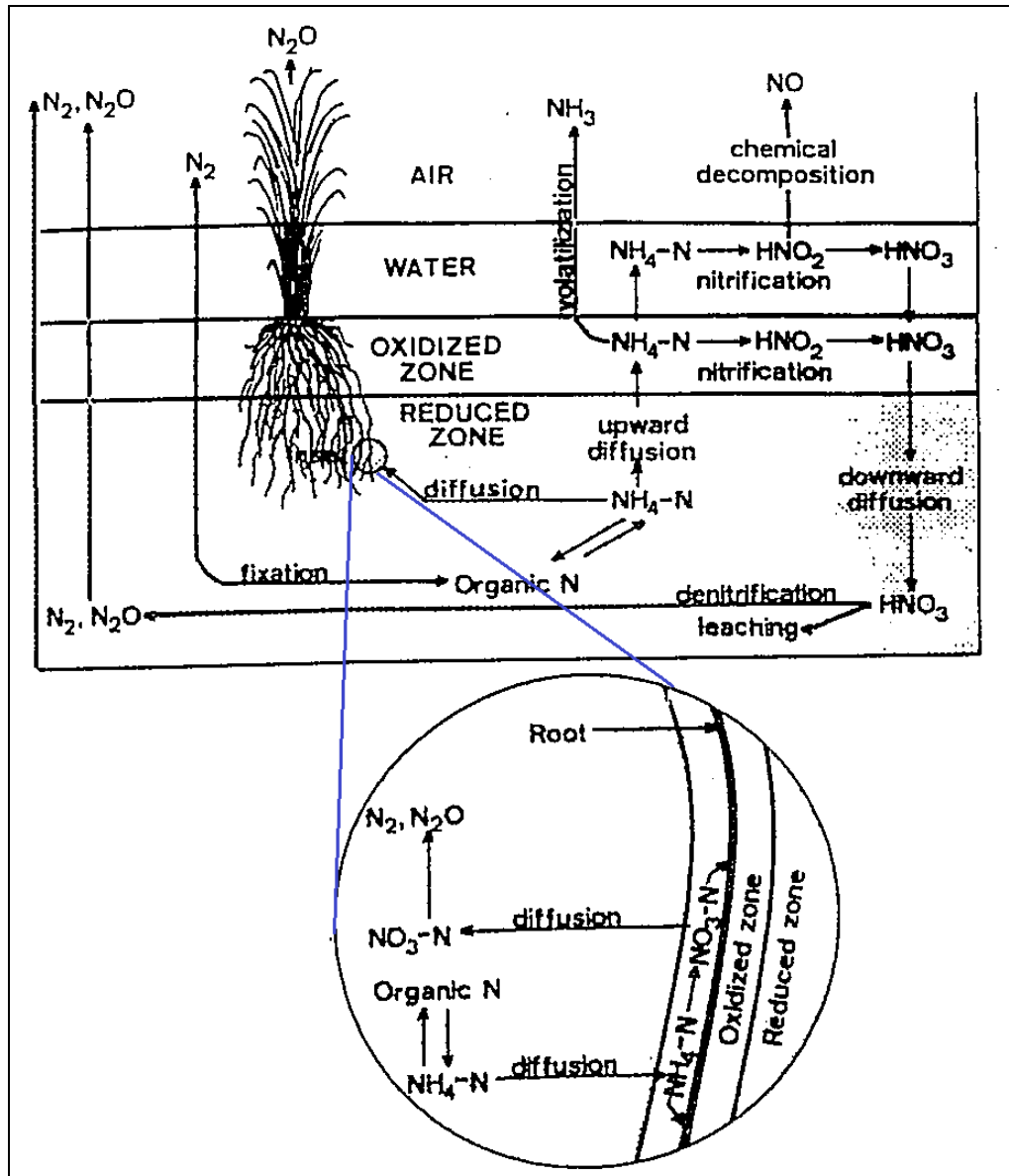


Fig. 8.1: Dominant processes involved in the formation of N_2O in soils: (I) Nitrification (oxidation of ammonium to nitrite and nitrate, or a biologically induced increase in the oxidation state of nitrogen and occurs under aerobic conditions) and (II) Denitrification (biological reduction of nitrate, NO_3^- or nitrite, NO_2^- , to nitrogen oxides or molecular nitrogen under anaerobic conditions). Adapted from Reddy (1982).

In the soil, N_2O can be formed by various processes depending on the amount of different forms of nitrogen, moisture level, soil temperature, and concentration of O_2 ,

ammonium and nitrate among other factors. Microbial de-nitrification and nitrification are the key dominant processes of N₂O within the natural N cycle (Smith, 1997; Bremner, 1997; Smith et al., 1998; Flessa et al., 2002; Towprayoon et al., 2005). Fine details about other minor sources of N₂O were clearly outlined in Bremner (1997).

It is thought that denitrification can be either a source or a sink for N₂O, which can either result in the formation of N₂O (as an intermediate byproduct), or consumption of N₂O, depending on environmental conditions such as O₂ levels, N levels, pH, and temperature (Smith, 1997). Under continuously flooded conditions, such as considered in this research most of the N₂O is produced by de-nitrification.

Attempts to quantify the direct effect of elevated soil temperature on N₂O emissions through soil warming studies have not produced a clear conclusion. In some studies, weak and negative correlations between N₂O fluxes and soil temperatures were reported, while in other studies, positive strong correlations were found. Large temporal variations of flux rates is one key source of error in determining the Q₁₀ of N₂O fluxes from cultivated soils (Flessa et al. 2002). Khamp et al. (1998) suggested that elevating temperatures leads to both positive and negative effects controlling N₂O emissions, and the overall effect results in small changes in N₂O release. It is evident that the effect of soil temperature on N₂O is complex, and requires further studies to get a full understanding of this feedback. Results from various studies indicate that the overall effect of temperature on N₂O emissions is generally positive, and that this rate of increase would be steeper when denitrification is more dominant than nitrification

(Smith, 1997). Two reasons are thought to be responsible for this observation, (I) increased soil respiration, which increases the anaerobic volume in which denitrification takes place, and (II) increased denitrification rate per unit anaerobic volume due to direct effect of temperature increase (Smith, 1997). Here the sensitivity of N₂O to soil temperature was investigated under carefully controlled soil warming experiments in order to quantify this positive feedback based on the IPCC global warming projections.

8.2 Aim and Objectives

The aim of this study was to determine whether global warming would result in increased N₂O emissions from rice paddies into the atmosphere. The objective of this research was to determine the Q₁₀s of N₂O production and fluxes under controlled greenhouse experiments in continuously flooded rice microcosms.

8.3 Materials and Methods

From the same experimental set-ups and samples to determine the effect of temperature on CH₄ (outlined in the preceding chapters), N₂O emissions and production rates were also determined from experiments described in Chapter 2. N₂O fluxes and its pore water concentrations were measured every three days and weekly, respectively. The collected samples were analyzed with a GC, equipped with an electron capture detector (ECD). N₂O fluxes were calculated using a linear regression of the observed concentrations with sampling time. Production rates and N₂O pore water concentrations were

determined using the approach outlined in Chapter 3 for determining CH₄ production rates and pore water concentrations, respectively.

8.4 Results and Discussion

8.4.1 N₂O Fluxes

With the exception of the increase after N-fertilization, N₂O fluxes were generally low for all temperature treatments. The high N₂O fluxes that were observed coincided with periods of N-fertilization, and diminished to almost zero afterwards. Similar results have been reported in many experiments (Yang et al., 2003; Ghosh et al., 2003; Lai, 2000). In particular, Gosh et al. (2003) found two sharp peaks in N₂O emissions just after fertilizer application, one at 2-5 DAT which gradually decreased and a new high during 15-33 DAT. Thereafter, the emissions were zero till 105 DAT for all the treatments. In contrast to higher second N₂O peaks than the first from their results, our second peaks were consistently lower than the first in all the treatments. The differences in the size of peaks reported in the former studies and those reported herein, could be due to dry spells reported in their results.

Under continuously flooded conditions, the N₂O emission into atmosphere is suppressed by the pressure of standing water and therefore most of the N₂O produced gets denitrified to N₂ within the soil, whilst under drier conditions, nitrification produces most of the N₂O. If the soil is flooded again, denitrifying bacteria will act on the nitrate released by nitrification to produce more N₂O (Ghosh et al., 2003).

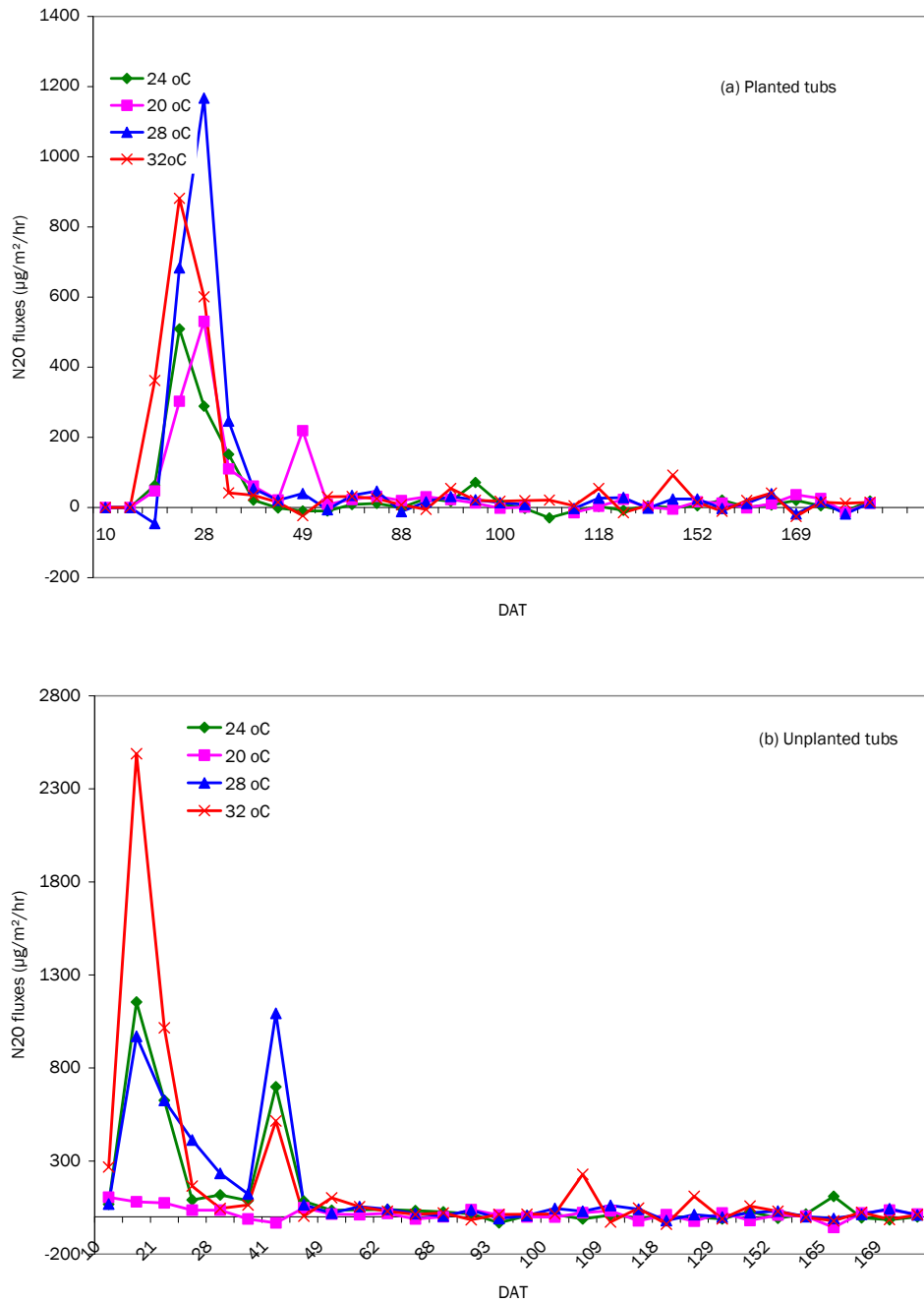


Fig. 8.2: N₂O emissions from both (a) planted and (b) unplanted tubs. Higher fluxes occurred in unplanted than in planted tubs, and the peaks coincide with episodes of fertilization. Elsewhere, the fluxes were nearly close to zero or slight negative. The impact of temperature was more apparent in unplanted tubs than in planted ones.

High temporal and spatial variability in N₂O fluxes was observed both within and between treatments. Two weeks after DAT, fluxes were low and highly variable, with negative N₂O fluxes occurring on many occasions. The observation was reported in various studies (McHale et al., 1998; Bremer, 1996). N₂O fluxes were two times higher in unplanted than in planted tubs.

A comparison of N₂O emissions from planted and unplanted tubs indicated that higher fluxes were consistently found in unplanted than planted tubs. In particular, highest peaks of N₂O fluxes were associated with the 28 °C treatment, whilst in unplanted tubs, the highest fluxes occurred in the 32 °C treatment. Two possibilities could account for these differences, either the N-fertilizer in the planted tubs was quickly used up by the plants at 32 °C, or the diffusion was very high at 32 °C in unplanted tubs. Either way, the presence of plants seemed to have played a part in the observed differences.

8.4.2 Effect of Soil Temperature on Fluxes

Within the 20-32 °C temperature range considered in our research, the effect of temperature was only evident a short period after N-fertilizer application. From other studies the results have been mixed-up. For example, McHale et al. (1998) did not find any significant relationship between N₂O and soil temperature. Because of the low emission rates when no N-fertilizer was applied, it would seem as though temperature did not have an effect on N₂O emissions. To observe the real effect of temperature, only the time averaged N₂O flux during the peaks were considered (Fig. 8.3).

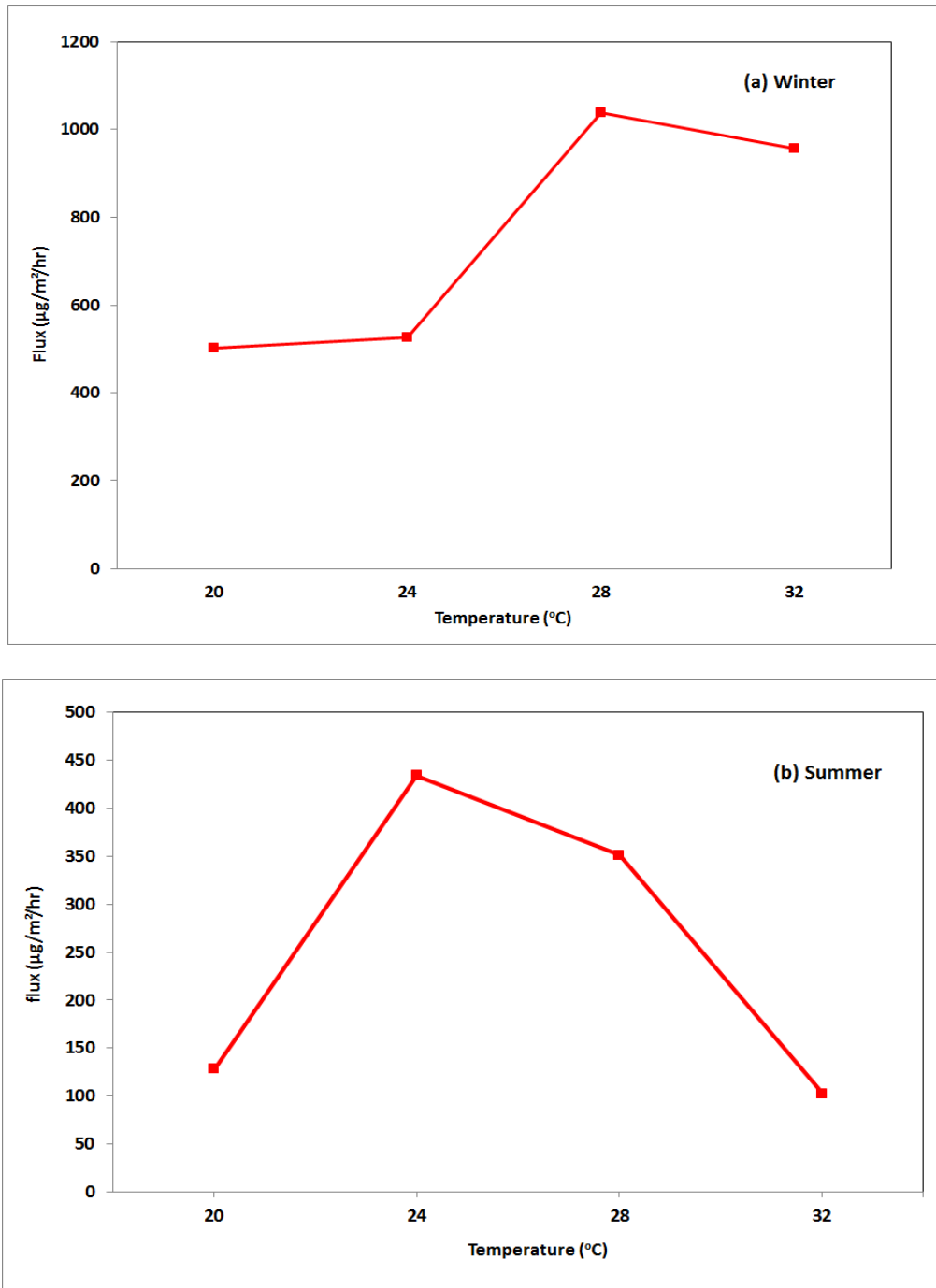


Fig. 8.3: Effect of soil temperature on N₂O fluxes during (a) Winter and (b) Summer.

Barnard et al. (2005) found that soil warming did not have large direct effects on N₂O emissions in the field.

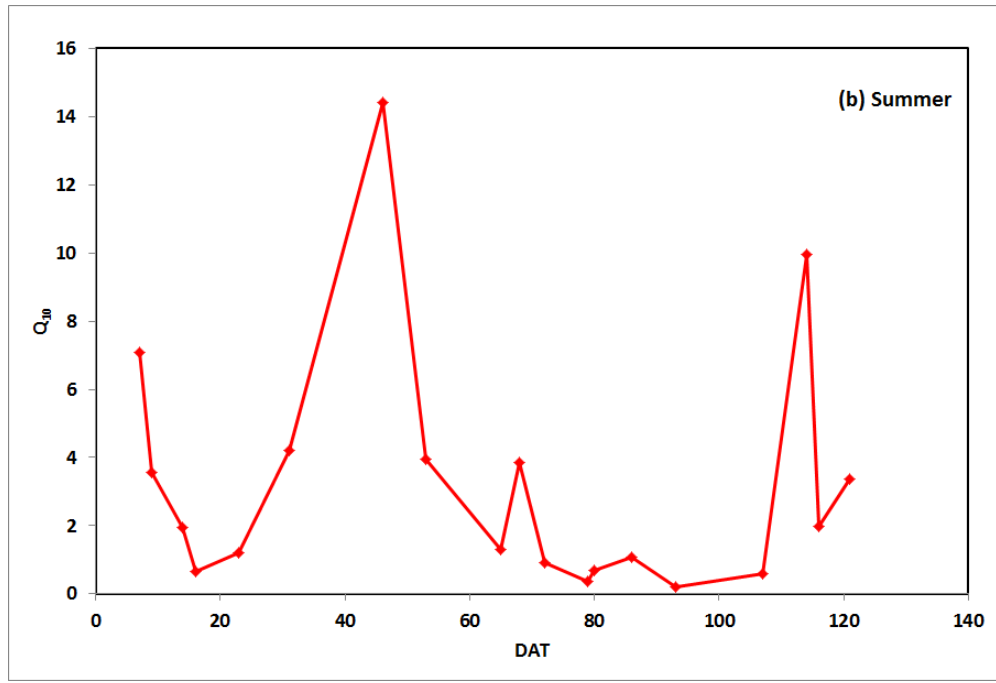
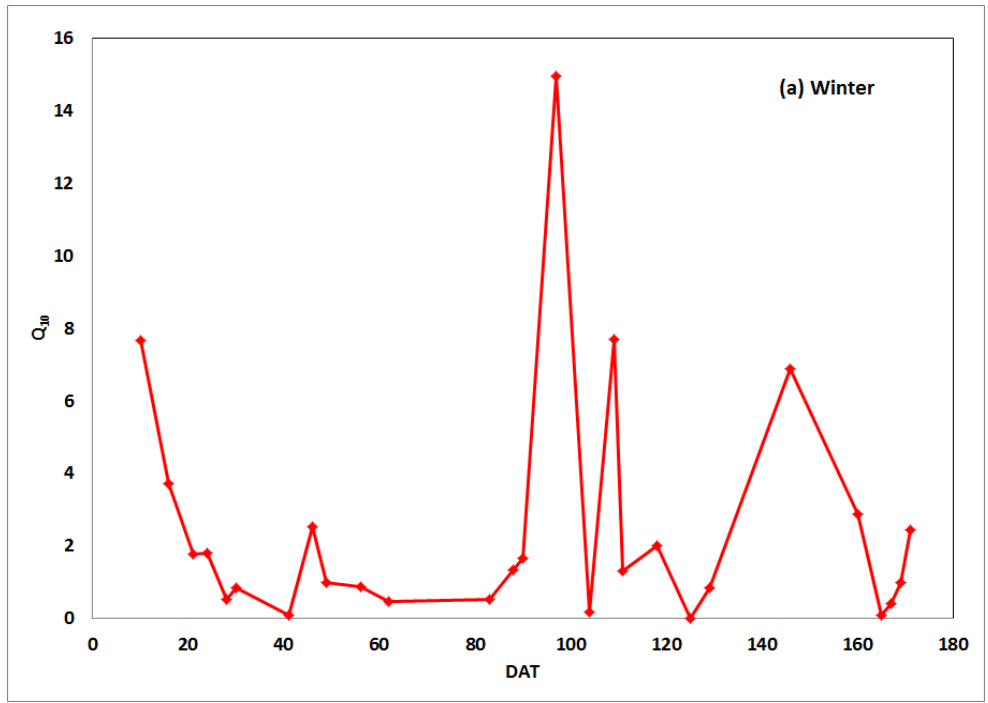


Fig. 8.3: Effect of soil temperature on fluxes as indicated by Q_{10} values. No discrepancy was observed between the data from (a) Winter and (b) Summer.

A further test on the effect of temperature was done by calculating the Q_{10} of the N_2O fluxes. The Q_{10} values for fluxes from both seasons ranged between 0.5 and 3.3. The very high values are thought to be associated with de-nitrification (Smith, 1997). Q_{10} values of between 0.4 and 9.4 were found in other studies (Koponen, 2006; Flessa et al., 2002). Slightly higher values (up to 15) on the upper limit of the Q_{10} were found by Smith et al. (1998).

8.4.3 Effect of Temperature on N_2O Production Rates

High production rates coincided with periods of fertilization. The production rate was high at the start of the season and almost zero elsewhere. Distribution was similar to that for N_2O fluxes. Q_{10} values varied between 0.2 and 1.8 (Fig. 8.4). These results are comparable to those from other studies. In particular, Q_{10} s ranging between 0.9 and 3.4 have been measured for N_2O production from forest soils (Castaldi, 2000). The highest Q_{10} s for N_2O have been associated with denitrification activity, such that a temperature rise enhances the activity of denitrifiers and increases the volume of the anaerobic soil fraction, where denitrification occurs (Smith, 1997).

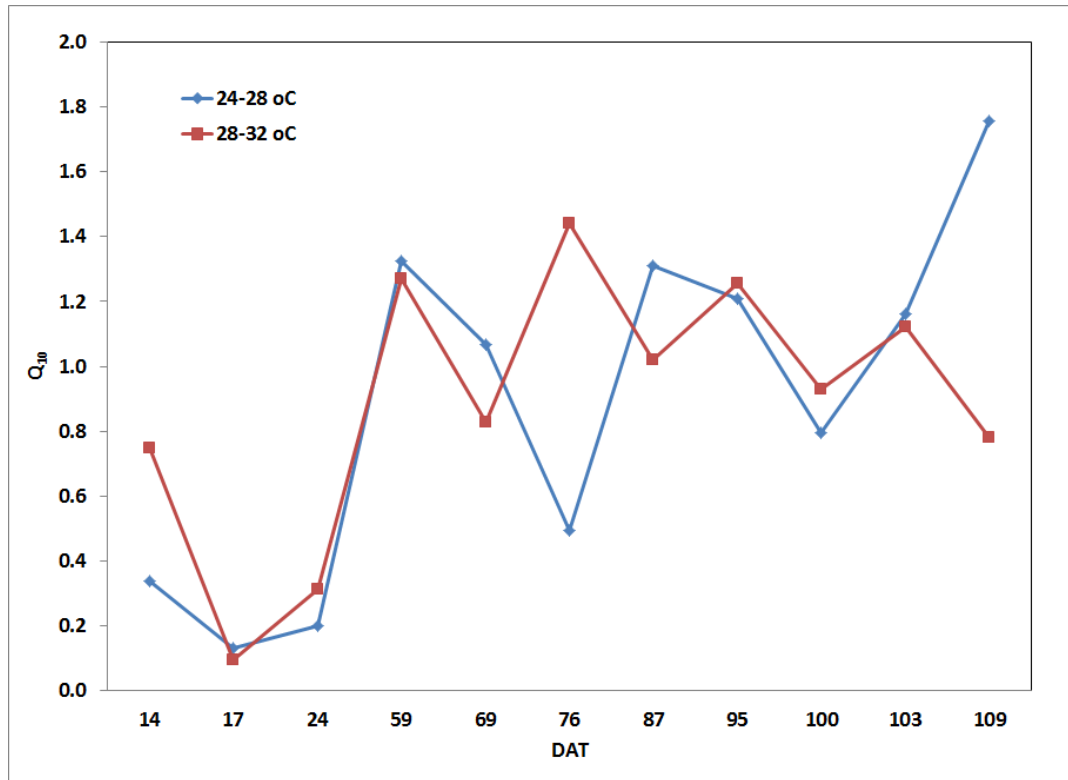


Fig. 8.4: The Q_{10} s of N_2O were fairly lower than those from fluxes. The values are independent of DAT, and varied from 0.1 to 1.8. Noteworthy were values below 1, indicative of decreasing production rates with elevated temperature.

Considering that our experiments were performed under continuously flooded conditions, denitrification was the major source of N_2O . From similar studies, on the effect of temperature on denitrification rate from nine soils (15 to 45 °C), the Q_{10} was approximately 2 (Stanford et al., 1975).

8.4.3 Pore Water Concentrations

Highest concentration of N_2O occurred at lowest temperature (20 °C), and lowest concentration at 32 °C. Increasing soil temperature reduces the pore water N_2O

concentration in the rhizosphere. Within all treatments, unplanted (control) tubs had the highest N₂O pore water concentrations.

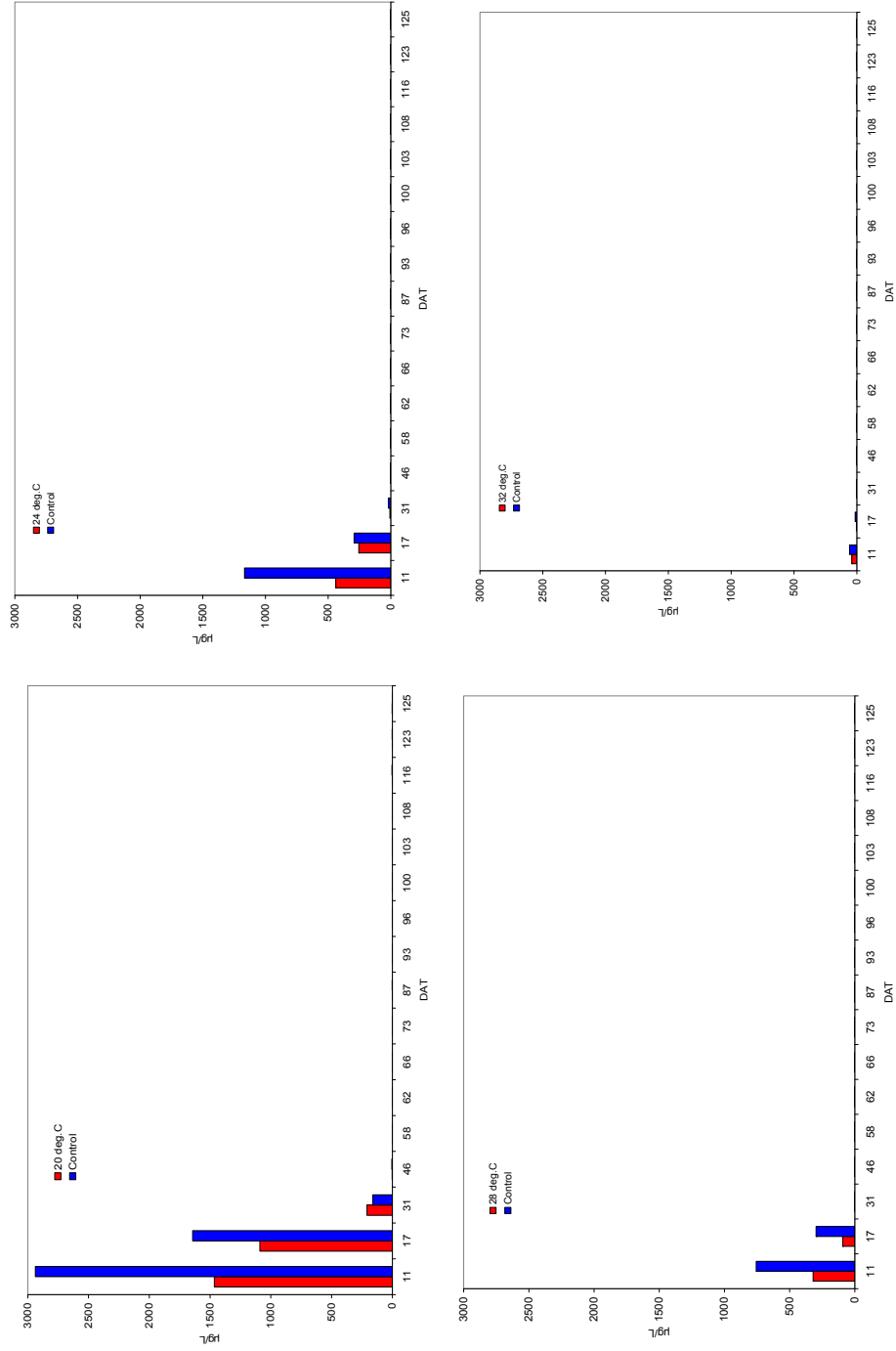


Fig. 8.5: Average pore water concentrations of N₂O with increasing soil temperature in the rhizosphere. The differences could be due to plant transport (increased transport through rice) and N utilization by plants.

The differences in N₂O concentrations could be due to a combination of fast transport and plant nitrogen uptake in planted tubs resulting in the depletion of nitrogen compounds that could be used for N₂O formation. Pore water concentrations were lower in higher soil temperature treatments than lower ones. For example, in both seasons, the N₂O concentration at 20 °C was almost 15 times higher than that at 32 °C.

8.5 Conclusion

The direct effects of temperature on N₂O emissions and production were successfully quantified. Spikes of N₂O emissions coincided with period of nitrogen fertilizer application. Both N₂O emission and production rates were dependent on soil temperature, with higher Q₁₀ values for fluxes than production. This may be the reason why very high N₂O fluxes occurred just after flooding, and diminishing to zero elsewhere. The Q₁₀ values found in our research, which are consistent with results from previous experiments and other studies, indicate that global warming will have a direct positive feedback on N₂O production and emission rates in flooded rice paddies. Nevertheless, the quantity and distribution of future N₂O emissions will depend on other indirect effects of global warming, such as the regional distribution of soil water content (water tables), and precipitation patterns.

Chapter 9

Summary and General Conclusion

This dissertation analyzed the potential impact of global warming on CH₄ and N₂O emission from agriculture. A two-year experimental set-up was carried out in the PSU Research Greenhouse, Portland State University, to determine the effect of global warming on CH₄ and N₂O emissions from rice agriculture. Throughout the sampling period, the rice microcosms were continuously flooded and heated using water baths, and only chemical fertilizer was used. This study fully captured the interdependences between temperature and production, oxidation and emissions. The study required the design and development of experiments from which simultaneous measurements of the Q_{10S} of CH₄ and N₂O would be determined. Six experiments were designed and conducted to determine effect of elevated soil temperature on CH₄ emission, production and oxidation. The research also involved determining the temperature sensitivity of N₂O, with special focus placed on production, emission and pore water concentrations. The research includes a study of the populations of methanogens and methanotrophs under different temperatures.

This study showed that temperature is an important factor for CH₄ emissions from agriculture, and that global warming will result in increased emissions. The magnitude of this feedback is potentially large enough to offset the present atmospheric CH₄ concentrations. However, this increase was most evident for soil temperatures below 30 °C. Beyond this temperature, fluxes decreased with increasing temperature. Within the

root zone, CH₄ concentration was only highly dependent on soil temperature during the start of the growing season, with highest concentration in the 10-15 cm soil horizon. Plants CH₄ transport capacity was the predominant limiting factor for fluxes during entire plant growth phase. The rate of CH₄ production was highly dependent on both soil temperature and time. The seasonally averaged Q₁₀ of CH₄ emission, production and oxidation were 2.0, 2.6 and 3.5, respectively. The population of methanotrophs was more sensitive to changes in soil temperature than methanogens, with seasonally averaged Q₁₀ values of 3.8 compared to 2.6 for methanogenesis. The implication of these results is that global warming is likely to promote CH₄ oxidation, and hence reduce the rate of increase of emissions from rice agriculture. Higher Q₁₀ values were also observed during the early part of the season than elsewhere. During this period of the season, rice plants were not well established, and ebullition was the only major CH₄ transport from the flooded rice tubs into the atmosphere. Since the ebullition is the major transport in wetlands, and that the processes of CH₄ formation and emission are similar those in natural wetlands, which are the largest contributors to atmospheric methane concentration, this positive temperature feedback is likely to contribute a significant additional CH₄ increase to the present atmospheric budget if the current global warming trend persists over the next century. Nevertheless, the magnitude of this impact will vary from one region to another due to the spatial variations in temperatures from one region to the other.

Weak regression coefficients were found between N₂O fluxes and soil temperature and low Q₁₀ values varying from 0.5 to 3.3 were observed. High N₂O production rates were

only evident during the period following fertilization. During other sampling periods, fluxes were mostly negative and the pore water concentration of N₂O was almost zero. Hence, N₂O emissions were mainly controlled by the availability of suitable substrates such as nitrogen compounds. The results also indicated that under global warming, the direct impact of temperature is not likely to be the major driver of N₂O emission increase from continuously flooded rice paddies in future. However, since moisture content is known to be one of the major factors that regulate N₂O emissions from agricultural soils, the pattern and distribution of these emissions will mostly depend on how much global warming will change the factors that affect the regional patterns and distributions of soil wetness. Such factors include water resources (rain, water tables) and water management practices.

While the current research has dealt with the impact of elevated soil temperatures on CH₄ and N₂O, future studies are required to determine the Q₁₀ of transport and the transport time of both CH₄ and N₂O, possibly by using isotopic experiments. Since on many occasions negative N₂O fluxes were observed during sampling, additional research is required to quantify how global warming will affect the uptake of N₂O in agricultural soils. Integrating the results of temperature sensitivities for CH₄ and N₂O for natural wetlands and rice soils into a global model to determine the likely impacts of global warming is also an area for further research, but may require determining the Q₁₀ factors for other ecosystems such as natural wetlands. The use of these models could improve the accuracy of the estimates of these emissions under a changing climate.

Such a process will require putting into consideration additional input factors such as future rainfall patterns and distribution of surface water resources.

References

- Abdalla, M., Jones, M., Smith, P. and Williams, M. (2009), Nitrous oxide fluxes and denitrification sensitivity to temperature in Irish pasture soils. *Soil Use and Management*, 25: 376–388.
- Abichou T., D. Powelson, J. Chanton, S. Escoriaza and J. Stern (2006). Characterization of methane flux and oxidation at a solid waste landfill. *Environmental Engineering*, 132(2): 220-228.
- Abichou T., J. Chanton, D. Powelson, J. Fleiger, S. Escoriaza, Y. Lei and J. Stern (2006). Methane flux and oxidation at two types of intermediate landfill covers. *Waste Management*, 26: 1305-1312.
- Alberto M.C.R., J.R.M. Arah, H.U. Neue, R. Wassmann, R.S. Lantin, J.B. Aduna, K.F. Bronson (2000). A sampling technique for the determination of dissolved methane in soil solution. *Chemosphere: Global Change Science*, 2: 57-63.
- Allen Jr, L.H., S.L. Albrecht, W. Colo, n-Guasp, S.A. Covell, J.T. Baker, D. Pan, and K.J. Boot (2003). Methane emissions of rice increased by elevated carbon dioxide and temperature. *Environmental Quality*, 32: 1978–1991.
- Aulakh M.S., R. Wassmann, C. Bueno and H. Rennenberg (2001). Impact of root exudates of different cultivars and plant development stages of rice (*Oryza sativa L.*) on methane production in a paddy soil. *Plant and Soil*, 230: 77-86.
- Auman A.J., Stolyar, S., Costello, A.M., and Lidstrom, M.E. (2000). Molecular characterization of methanotrophic isolates from freshwater lake sediment. *Applied and Environmental Microbiology*, 66(12): 5259-5266.
- Bergamaschi, P. (1997). Seasonal variations of stable hydrogen and carbon isotope ratios in methane from a Chinese rice paddy. *Geophysical Research*, 102(D21): 25383-25393.
- Bharati, K., S.R. Mohanty, V.R. Rao, T.K. Adhya (2001). Influence of flooded and non-flooded conditions on methane flux from two soils planted to rice. *Chemosphere - Global Change Science*, 3: 25 - 32.
- Bodelier P.L.E. and H.J. Laanbroek (2004). Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiology Ecology*, 47: 265-277.
- Bodelier, P.L.E., and Frenzel, P. (1999). Contribution of methanotrophic and nitrifying bacteria to CH₄ and NH₄⁺ oxidation in the rhizosphere of rice plants as determined by new methods of discrimination. *Applied and Environmental Microbiology*, 65:1826-183.

- Bodelier, P.L.E., Roslev, P., Henckel, T. and Frenzel, P. (2000). Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature*, 403: 421-424.
- Borges, R. and Chaney, W. (1989). Root temperature affects mycorrhizal efficacy in *Fraxinus pennsylvanica* Marsh. *New Physiology*, 112: 411-417.
- Bosse, U., and Frenzel, P. (1997). Activity and distribution of methane-oxidizing bacteria in flooded rice soil microcosms and in rice plants (*Oryza sativa*). *Applied and Environmental Microbiology*, 63:1199-1207.
- Bremner, J.M. (1997). Sources of nitrous oxide in soils. *Nutrient Cycling in Agroecosystems*, 49: 7–16.
- Byrne, B.H., E.R. Austin and B.K. Tays. Methane emissions from flooded rice soils and plants under controlled conditions. *Soil Biology and Biochemistry*, 27(3): 331 - 339. 1995.
- Cabral A.R., M.A. Capanema, J. Gebert, J.F. Moreira and L.B. Jugnia (2010). Quantifying microbial methane oxidation efficiencies in two experimental landfill biocovers using stable isotopes. *Water, Air and Soil Pollution*, 209: 157–172.
- Castaldi S., Smith K.A. (1998). The effect of different N substrates on biological N₂O production from forest and agricultural light textured soils. *Plant Soil*, 199: 229–238.
- Castaldi, S. (2000). Responses of nitrous oxide, dinitrogen and carbon dioxide production and oxygen consumption to temperature in forest and agricultural light-textured soils determined by model experiment. *Biology and Fertility of Soils*, 32: 67–72.
- Chanton, J.P., G.J. Whiting, N.E. Blair, C.W. Lindau, and P.K. Bollich (1997). Methane emission from rice: Stable isotopes diurnal variations and CO₂ exchange. *Global Biogeochemical Cycles*, 11(1): 15-27.
- Chapuis-Lardy L., A. Metay, M. Martinet, M. Rabenarivo, J. Toucet, J.M. Douzet, T. Razafimbelo, R.L. Rabeharisoa, J. Rakotoarisoa (2009). *Geoderma*, 148(3-4): 421-427.
- Chasar L.S., J.P. Chanton, P.H. Glaser and D.I. Siegel (2000). Methane concentration and stable isotope distribution as evidence of rhizospheric processes: comparison of a fen and bog in the glacial lake Agassiz peatland complex. *Annals of Botany*, 86: 655-663.

- Cheng, X., Y. Luo, Q. Xu, G. Lin, Q. Zhang, J. Chen and B. Li (2010). Seasonal variation in CH₄ emission and its ¹³C-isotopic signature from *Spartina alterniflora* and *Scirpus mariqueter* soils in an estuarine wetland. *Plant Soil*, 327: 85–94.
- Chidthaisong, A., K-J. Chin, D.L. Valentine, And S.C. Tyler (2002). A comparison of isotope fractionation of carbon and hydrogen from paddy field rice roots and soil bacterial enrichments during CO₂/H₂ methanogenesis. *Geochimica et Cosmochimica Acta*, 66(6): 983-995.
- Chin J-K., Lukow, T. and Conrad, R. (1999). Effect of temperature on structure and function of the methanogenic archaeal community in an anoxic rice field soil. *Applied Environmental Microbiology*, 65(6): 2341-2349.
- Cicerone R.J., J.D. Shetter and C.C. Delwiche. 1983. Seasonal variation of methane flux from a Californian rice paddy. *Geophysical Research*, 88: 11022-11024.
- Coleman, D.D., R.J. Bruno and S. Martin (1981). Fractionation of carbon and hydrogen isotopes by methane-oxidizing bacteria. *Geochimica et Cosmochimica Acta*, 45: 1033-1037.
- Conrad R. (2002). Control of microbial methane production in wetland rice fields. *Nutrient Cycling in Agroecosystems*, 64: 59–69.
- Conrad, R. (1996). Soil Microorganisms as Controllers of Atmospheric Trace Gases (H₂, CO, CH₄, OCS, N₂O, and NO). *Microbiological Reviews*, 60(4): 609–640.
- Conrad, R. 1999. Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediment. *FEMS Microbiology and Ecology*, 28: 193-202.
- Conrad, R., M. Klose, P. Claus (2002). Pathway of CH₄ formation in anoxic rice field soil and rice roots determined by ¹³C-stable isotope fractionation. *Chemosphere*, 47: 797-806.
- Crill et al. (1994). Temperature and N fertilization effects on methane oxidation in a drained peatland soil. *Soil Biology and Biochemistry*, 26 (10): 1331-1339.
- Damgaard, L.R., N.P. Revsbech, and W. Reichardt (1998). Use of an oxygen-insensitive microscale biosensor for methane to measure methane concentration profiles in a rice paddy. *Applied and Environmental Microbiology*, 864 - 870.

- Davidson EA, Swank WT, Perry TO (1986). Distinguishing between nitrification and denitrification as a source of gaseous nitrogen production in soil. *Applied and Environmental Microbiology*, 52: 1280–1286.
- Davidson, E.A. and I.A. Janssens. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature Reviews*, 440: 165 - 173.
- Dawson, T.E., S. Mambelli, A.H. Plamboeck, P.H. Templer and K.P. Tu (2002). Stable isotopes in plant ecology. *Annual Reviews of Ecological Systems*, 33: 507-59.
- Deleens, E., I. Treichel and M.H. Otleary (1985). Temperature dependence of carbon isotope fractionation in CAM plants. *Plant Physiology*, 79: 202-206.
- Ding, W., Z. Cai and H. Tsuruta (2004). Diel variation in methane emissions from the stands of *Carex lasiocarpa* and *Deyeuxia angustifolia* in a cool temperate freshwater marsh. *Atmospheric Environment*, 38:181–188
- Dörsch P., A. Palojarvi and S. Mommertz. Overwinter greenhouse gas fluxes in two contrasting agricultural habitats (2004). *Nutrient Cycling in Agroecosystems*, 70: 117–133.
- Farquhar, G.D., M.H. O'Leary and J.A. Berry (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, 9: 121-137.
- Fey A., P. Claus and R. Conrad (2004). Temporal change of ¹³C-isotope signatures and methanogenic pathways in rice field soil incubated anoxically at different temperatures. *Geochimica et Cosmochimica Acta*, 68(2): 293-306.
- Flessa H., R. Ruser, R. Schilling, N. Loftfield, J.C. Munch, E.A. Kaiser, F. Beese (2002). N₂O and CH₄ fluxes in potato fields: automated measurement, management effects and temporal variation. *Geoderma*, 105: 307–325.
- Freitag, T.E., Toet, S., Ineson, P. and Prosser, J. (2010). Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog. *FEMS Microbiology and Ecology*, 73: 157-165.
- Gal'chenko, V.F. (2004). On the problem of anaerobic methane oxidation. *Microbiology*, 73 (5): 599-608. Translated from *Mikrobiologiya*, 73(5): 698-707.
- Gauci, V., D. Fowler, S.J. Chapman and N.B. Dise (2004). Sulfate deposition and temperature controls on methane emission and sulfur forms in peat. *Biogeochemistry*, 71: 141–162.

- Ghosh S., D. Majumdar, M.C. Jain (2003). Methane and nitrous oxide emissions from an irrigated rice of North India. *Chemosphere*, 51: 181-195.
- Gilbert, B., and Frenzel, P. (1995). Methanotrophic bacteria in the rhizosphere of rice microcosms and their effect on porewater methane concentration and methane emission. *Biology and Fertility of Soils*, 20: 93-100.
- Goevert, D. and R. Conrad (2009). Effect of substrate concentration on carbon isotope fractionation during acetoclastic methanogenesis by *Methanosarcina barkeri* and *M. acetivorans* and in rice field soil. *Applied and Environmental Microbiology*, 75(9): 2605-2612.
- Gómez, K.E., G. Gonzalez-Gil, A. Lazzaro and M.H. Schroth (2009). Quantifying methane oxidation in a landfill-cover soil by gas push-pull tests. *Waste Management*, 29: 2518-2526.
- Gonfiantini, R., Stichler, W., and Rozanski, K. (1995). Standards and intercomparison materials distributed by the International Atomic Energy Agency for stable isotope measurements, in Reference and intercomparison materials for stable isotopes of light elements, Vienna, Austria, International Atomic Energy Agency, IAEA-TECDOC-825, 13-29.
- Goulding, J.L. Monteith, V.R. Phillip (1995). The measurement of nitrous oxide emissions from soil by using chambers [and discussion]. *Philosophical Transactions of Royal Society*, 351: 327-338.
- Gross M.F., M.A. Hardisky, P.L. Wolf and V. Klemas (1993). Relationship among *Typha* biomass, pore water methane, and reflectance in a Delaware (U.S.A.) brackish marsh. *Coastal Research*, 9(2): 339 - 355.
- Grossman, E.L. (2002). Stable carbon isotopes as indicators of microbial activity in aquifers, in Manual of Environmental Microbiology, 2nd ed., C.J. Hurst et al. (eds.), American Society for Microbiology Press, Washington, DC, p. 728-742.
- Neue H.U. and P.A. Roger (1994). Potential of methane emission in major rice ecologies. In: Zepp RG (ed) *Climate Biosphere Interaction*, New York: Wiley and Sons, p 65-93.
- Hanson, R.S., and Hanson, T.E. 1996. Methanotrophic bacteria. *Microbiology Reviews*, 60: 439-471.
- Harden H.S. and J.P. Chanton (1994). Locus of methane release and mass-dependent fractionation from two wetland macrophytes: *Limnology and Oceanography*, 39(1): 148-154.

- Helmut Schutz, Wolfgang Seiler and Ralf Conrad (1990). Influence of soil temperature on methane emission from rice paddy fields. *Biogeochemistry*, 11: 77-95.
- Heninger. R.L. and White, D.P. (1974). Tree seedling growth at different soil temperatures. *Forest Science*, 20: 363-367.
- Henn, M.R., G. Gleixner and I.H. Chapela (2002). Growth-dependent stable carbon isotope fractionation by Basidiomycete fungi: pattern and physiological process. *Applied and Environmental Microbiology*, 4956-4964.
- Hinrichs, K.-U., A. Boetius (2002). The anaerobic oxidation of methane: new insights in microbial ecology and biogeochemistry. From Wefer G, Billett D, Hebbeln D, Jørgensen BB, Schlüter M., Van Weering T (eds). *Ocean Margin Systems. Springer-Verlag Berlin Heidelberg*, 457-477.
- Holler, T., G. Wegener, K. Knittel, A. Boetius, B. Brunner, M.M. Kuypers, F. Widdel (2009). Substantial $^{13}\text{C}/^{12}\text{C}$ and D/H fractionation during anaerobic oxidation of methane by marine consortia enriched in vitro. *Environmental Microbiology Reports*, 1(5): 370 - 376.
- Holzappel-Pschorn, A., R. Conrad, and W. Seiler. 1985. Production, oxidation and emission of methane in rice paddies. *FEMS Microbiological Ecology*, 31: 343-351.
- Hosono, T. and I. Nouchi (1997). The dependence of methane transport in rice plants on the root zone Temperature. *Plant and Soil*, 191: 233-240.
- Hou A.X., G.X. Chen, Z.P. Wang, O. Van Cleemput, and W.H. Patrick, Jr (2000). Methane and nitrous oxide emissions from a rice field in relation to soil redox and microbiological processes. *Soil Science Society American Journal*, 64:
- Huang, Y., R.L. Sass, F.M. Fisher, Jr (2002). A semi-empirical model of methane emission from flooded rice paddy soils. *Global Change Biology*, 4(3): 247 - 268.
- Hutton, W.E and Zobell C.E. 1949. The occurrence and characteristics of methane-oxidizing bacteria in marine sediments. *Bacteriology*, 58(4): 463-473.
- Insam, H. and B. Wett (2008). Control of GHG emission at the microbial community level. *Waste Management*, 28: 699-706.
- IPCC (1996). *Climate Change 1995: The science of climate change*. Cambridge University Press.

- IPCC (2007a). Couplings between changes in the climate system and biogeochemistry. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Solomon et al. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- IPCC (2007b). Changes in atmospheric constituents and in radiative forcing. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Solomon et al. (eds.)].
- IPCC (2007c). Global climate projections. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Solomon et al. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2007.
- IPCC (2007d). Agriculture. In *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Metz et al. (eds.)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jiang C., Wang Y., Zheng X., Zhu B., Huang Y., And Hao Q. (2006). Methane and Nitrous Oxide Emissions from Three Paddy Rice Based Cultivation Systems in Southwest China. *Advances in Atmospheric Sciences*, 23(3): 415 - 424.
- Joulian C., Escoffier, S., Mer, J.L., Neue, H.-U. and Roger, P.A. (1997). Populations and potential activities of methanogens and methanotrophs in rice fields: relations with soil properties. *European Journal of Soil Biology*, 33 (2): 105-116.
- Kamp T., H. Steindl, R.E. Hantschel, F. Beese J.-C. Munch (1998). Nitrous oxide emissions from a fallow and wheat field as affected by increased soil temperatures. *Biology and Fertility of Soils*, 27: 307- 314.
- Khalil M.A.K., C. Butenhoff, A.Sithole and M.J. Shearer. The feedback of climate change on methane emissions from terrestrial ecosystems. In preparation.
- Khalil, M.A.K. and R.A. Rasmussen, M.-X. Wang and L. Ren (1991). Methane emissions from rice fields in China. *Environmental Science and Technology*, 25: 979-981.
- Khalil, M., Rasmussen, R., Shearer, M., Dalluge, R., Ren, L. and Duan. C.-L. (1998). Factors affecting methane emissions from rice fields. *Geophysical Research*, 103(D19): 25219-25231.

- Khalil, M.A.K., M.J. Shearer, R.A. Rasmussen, Li Xu, and Jin-Luan Liu (2008). Methane and nitrous oxide emissions from subtropical rice agriculture in China. *Geophysical Research*, 113, G00A05, doi:10.1029/2007JG000462.
- Khalil, M.A.K. and Shearer M. (1993a). Sources of methane: overview. In M.A.K. Khalil (ed.). *Atmospheric methane: sources, sinks and role in global change*. Springer-Verlag, Berlin Germany, Chapter 10, pp 180-198.
- Khalil, M.A.K. and Shearer M. (1993b). Rice agriculture: emissions. In M.A.K. Khalil (ed.). *Atmospheric methane: sources, sinks and role in global change*. Springer-Verlag, Berlin Germany, Chapter 12, pp 230-253.
- Khalil, M.A.K. I, M.J. Shearer, R.A. Rasmussen, Li Xu, and J-L. Liu (2008a). Methane and nitrous oxide emissions from subtropical rice agriculture in China. *Geophysical Research*, 113, G00A05, doi:10.1029/2007JG000462,.
- Khalil, M.A.K., M.J. Shearer, R.A. Rasmussen, D. Changlin, and L.X. Ren (2008b). Production, oxidation, and emissions of methane from rice fields in China. *Geophysical Research*, 113: G00A04, doi:10.1029/2007JG000461.
- Khalil, M.A.K., R.A. Rasmussen, M.J. Shearer, R.W. Dalluge, L.X. Ren and C-L. Duan. (1998b). Factors affecting methane emissions from rice fields. *Geophysical Research*, 103 (D19): 25 219-25 231.
- Khalil, M.A.K., R.A. Rasmussen, M.J. Shearer, R.W. Dalluge, L.X. Ren and C-L. Duan (1998c). Measurements of methane emissions from rice fields in China. *Geophysical Research*, 103 (D19): 25 181-25 210.
- Khalil, M.A.K., R.A. Rasmussen, M.J. Shearer, Z.L. Chen, Heng Yao, and J. Yang (1998a). Emissions of methane, nitrous oxide, and other trace gases from rice fields in China. *Geophysical Research*, 103 (D19): 25 241-25 250.
- Khalil, M.A.K., Rasmussen, R.A., and Shearer, M.J. (1989). Trends of atmospheric methane during the 1960s and 1970s. *Geophysical Research*, 94(D15): 18279-18288.
- Khalil, M.A.K., Rasmussen, R.A., Shearer, M.J. (1998e). Flux measurements and sampling strategies: applications to methane emissions from rice fields. *Geophysical Research*, 103(D19): 25211-25218.
- Kimura, M., minoda, and J. Murase (1993). Water soluble materials in paddy soil ecosystem. II Effects of temperature on contents of total organic materials, organic acids, and methane in leachate from submerged paddy soils amended with rice straw. *Soil Science and Plant Nutrition*, 39: 713 – 724.

- Kipphut G.W. and C.S. Martens (1982). Biogeochemical cycling in an organic-rich coastal marine basin-3. Dissolved gas transport in methane-saturated sediments. *Gwchimica Cosmochimica Acta*, 46: 2049 - 2060.
- Knittel, K. and A. Boetius (2009). Anaerobic oxidation of methane: progress with an unknown process. *Annual Reviews of Microbiology*, 63: 311-334.
- Kolb S., Knief, C., Stubner, S., and Conrad, R. (2003). Quantitative detection of methanotrophs in soil by novel pmoa-targeted real-time PCR assays. *Applied and Environmental Microbiology*, 69(5): 2423-2429.
- Koponen H.T., C.E. Durana, M. Maljanena, J. Hytonen, P.J. Martikainen (2006). Temperature responses of NO and N₂O emissions from boreal organic soil. *Soil Biology and Biochemistry*, 38: 1779–1787.
- Kreye C., K. Dittert, X. Zheng, X. Zhang, S. Lin, H. Tao, B. Sattelmacher (2007). Fluxes of methane and nitrous oxide in water-saving rice production in north China. *Nutrient Cycling in Agroecosystems*, 77: 293–304.
- Kumaraswamy S., B. Ramakrishnan and N. Sethunathan (2001). Wetlands and aquatic processes methane production and oxidation in an anoxic rice soil as influenced by inorganic redox species. *Environmental Quality*, 30: 2195-2201.
- Liblik, L.K., T.R. Moore, J.L. Bubier and S.D. Robinson (1997). Methane emissions from wetlands in the zone of discontinuous permafrost: Fort Simpson, Northwest Territories, Canada. *Global Biogeochemical Cycles*, 11(4): 485 – 494.
- Lindau, C.W. (1994). Methane emissions from Louisiana rice fields amended with nitrogen fertilizers. *Soil Biology and Biochemistry*, 26(3): 353 - 359.
- Lu, Y., R. Wassmann, H-U. Neue, C. Huang (1999). Impact of phosphorous supply on root exudation, aerenchyma formation and methane emission of rice plants. *Biogeochemistry*, 47: 203 – 213.
- Lu, Y., R. Wassmann, H-U. Neue, C. Huang (2000). Dynamics of dissolved organic carbon and methane emissions in a flooded rice soil. *Soil Science Society American Journal*, 64: 2011 - 2017.
- Luton, P.E., Wayne, J.M., Sharp, R.J. and Riley, P.W. (2002). The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogens populations in landfill. *Microbiology*, 148: 3521-3530.

- Macdonald et al. (1998). Methane emission rates from a northern wetland; response to temperature, water table and transport. *Atmospheric Environment*, 32(19): 3219 - 3227.
- Mariko, S., Y. Harazono, N. Owat and I. Nouchi (1991). Methane in flooded soil water and the emission through rice plants to the atmosphere. *Environmental and Experimental Botany*, 31(3): 343 – 350.
- Martens, C.S., D.B. Albert, and M.J. Alperin (1999). Stable isotope tracing of anaerobic methane oxidation in the gassy sediments of Eckernförde Bay, German Baltic Sea. *American J. of Science*, 299: 589-610.
- McCrackin, M.L. and J.J. Elser (2010). Atmospheric nitrogen deposition influences denitrification and nitrous oxide production in lakes. *Ecology*, 91: 528–539.
- McHale P.J., M.J. Mitchell, and F.P. Bowles (1998). Soil warming in a northern hardwood forest: trace gas fluxes and leaf litter decomposition. *Canadian J. Forestry Research*, 28: 1365–1372.
- Mer, J.L. and P. Roger (2001). Production, oxidation, emission and consumption of methane by soils: A review. *European J. of Soil Biology*, 37: 25-50.
- Minami, K. (1997). Atmospheric methane and nitrous oxide: sources, sinks and strategies for reducing agricultural emissions. *Nutrient Cycling in Agroecosystems*, 49: 203-211.
- Mitra S., R. Wassmann, M.C. Jain and H. Pathak (2002). Properties of rice soils affecting methane production potentials: 2. Differences in topsoil and subsoil. *Nutrient Cycling in Agroecosystems*, 64: 183–191.
- Mohanty S.R., Bodelier, P.L.E. and Conrad, R. (2007). Effect of temperature on composition of the methanotrophic community in rice field and forest soil. *FEMS Microbiology and Ecology*, 62: 24-31.
- Murase J., Y. Sakai, A. Sugimoto, K. Okubo, and M. Sakamoto (2003). Sources of dissolved methane in Lake Biwa. *Limnology*, 4: 91 - 99.
- Nakagawa, F., N. Yoshida, A. Sugimoto, E. Wada, T. Yoshioka, S. Ueda, P. Vijarnsorn. 2002. Stable isotope and radiocarbon compositions of methane emitted from tropical rice paddies and swamps in Southern Thailand. *Biogeochemistry*, 61(1): 1-19.
- Neff J.C. and G.P. Asner (2001). Dissolved organic carbon in terrestrial ecosystems: Synthesis and a model. *Ecosystems*, 4: 29 – 48.

- Neue H.-U. and P.A. Roger (1994). Potential of methane emission in major rice ecologies. In: Zepp R. G. (Ed.). *Climate biosphere interaction: biogenic emissions and environmental effects of climate change*. John Wiley and Sons, pp. 65-93.
- Neue, H. 1993. Methane emission from rice fields: Wetland rice fields may make a major contribution to global warming. *BioScience*, 43 (7): 466-473.
- O'Leary, M.H. (1981). Carbon isotopic fractionation in plants. *Phytochemistry*, 20: 553-567.
- O'leary, M.H., I. Treichel and M. Rooney (1986). Short-Term Measurement of Carbon Isotope Fractionation in Plants. *Plant Physiology*, 80: 578-582.
- Parashar, D.C., P.K. Gupta, J. Rai, R.C. Sharma and N. Singh (1993). Effect of soil temperature on methane emission from paddy fields. *Chemosphere*, 26(1-4): 247-250.
- Pastor, J., J. Solin, S.D. Bridgham, K. Updegraff, C. Harth, P. Weishampel and B. Dewey (2003). Global warming and the export of dissolved organic carbon from boreal peatlands. *Oikos*, 100: 380-386
- Peng J., Lu, Z., Rui, J. and Lu, Y. (2008). Dynamics of the methanogenic archaeal community during plant residue decomposition in an anoxic rice field soil. *Applied and Environmental Microbiology*, 74(9): 2894-2901.
- Peterjohn, W.T., J.M. Melillo, F.P. Bowles, P.A. Steudler (1993). Soil warming and trace gas fluxes: experimental design and preliminary flux results. *Oecologia*, 93: 18-24.
- Prieme, A. (1994). Production and emission of methane in a brackish and a freshwater wetland. *Soil biology and Biochemistry*, 26(I): 7-18.
- Rasmussen, R.A. and Khalil, M.A. (1981). Atmospheric methane: trends and seasonal cycles. *Geophysical Research*, 86: 9826-9832.
- Rath A.K., B. Ramakrishnan, N. Sethunathan (2002). Temperature dependence of methane production in tropical rice soils. *Geomicrobiology*, 19: 581-592.
- Reay, D.S., D.B. Nedwell (2004). Methane oxidation in temperate soils: effects of inorganic N. *Soil Biology and Biochemistry*, 36: 2059-2065.
- Reddy, K.R. (1982). Nitrogen cycling in a flooded-soil ecosystem planted to rice (*Oryza sativa L*). *Plant and Soil*, 67: 209 - 220.

- Reeburgh, W.S., A.I. Hirsch, F.J. Sansone, B. N. Popp, and T.M. Rust (1997). Carbon kinetic isotope effect accompanying microbial oxidation of methane in boreal forest soils. *Geochimica et Cosmochimica Acta*, 61(22): 4761-4767.
- Ritchie D.A., Edwards, C., Mcdonald, I.R. and Murrell, J.C. (1997). Detection of methanogens and methanotrophs in natural environments. *Global Change Biology*, 3: 339-350.
- Riveros-Iregui, D.A. and J.Y. King (2008). Isotopic evidence of methane oxidation across the surface water-ground water interface. *Wetlands*, 28(4): 928-937.
- Roslev P., Iversen, N. and Henriksen, K. (1997). Oxidation and assimilation of atmospheric methane by soil methane oxidizers. *Applied and Environmental Microbiology*, 63(3): 874-880.
- Rothfuss, F. and R. Conrad (1993). Vertical profiles of CH₄ concentrations, dissolved methanogenic substrates and processes involved in CH₄ production in a flooded Italian rice field. *Biogeochemistry*, 18: 137-152.
- Rothfuss, F., F.G.C. Bijnen, R. Conrad, F.J.M. Harren and J. Reuss (1996). Combination of photoacoustic detector with gas diffusion probes for the measurement of methane concentration gradients in submerged paddy soil. *Chemosphere*, 33(12): 2487 - 2504.
- S.C. Tyler, P.M. Grill, and G.W. Brailsford (1994). 13C/12C Fractionation of methane during oxidation in a temperate forested soil. *Geochimica et Cosmochimica Acta*, 58(6): 1625-1633.
- Sansone, F.J., M.E. Holmes, and B.N. Popp (1999). Methane stable isotopic ratios and concentrations as indicators of methane dynamics in estuaries. *Global Biogeochemical Cycles*, 13(2), 463-474.
- Sass R.L., F.M. Fischer, P.A. Harcombe, and F.T. Turner. 1991. Methane production and emission in a Texas rice field. *Global Biogeochemical Cycles*, 4: 47-68.
- Sass, R.L., F.M. Fisher, F.T. Turner, and M.F. Jund (1991). Methane emission from rice fields as influenced by solar radiation, temperature and straw incorporation. *Global Biogeochemical Cycles*, 5(4): 335-350.
- Satpathy, S.N., A.K. Rath, B. Ramakrishnan, V.R. Rao, T.K. Adhya and N. Sethunathan (1997). Diurnal variation in methane efflux at different growth stages of tropical rice. *Plant and Soil*, 195: 267-271.
- Segers R (1998). Methane production and methane consumption: A review of processes underlying wetland methane fluxes. *Biogeochemistry*, 41(1): 23 - 51.

- Schütz, H., A. Holzapfel-Pschorn, R. Conrad, H. Rennenberg, and W. Seiler. 1989. A 3-year continuous record on the influence of daytime, season and fertilizer treatment on methane emission rates from an Italian rice paddy field. *Geophysical Research*, 94: 16405-16416.
- Schutz, H., W. Seiler and R. Conrad (1990). Influence of soil temperature on methane emission from rice paddy fields. *Biogeochemistry*, 11: 11-95.
- Schutz, H., Wolfgang Seiler, Ralf Conrad (1989). Processes involved in formation and emission of methane in rice paddies. *Biogeochemistry*, 7(1): 33-53.
- Sebacher, D.I., Harriss R.C. and Bartlett K.B. (1985). Methane emissions to the atmosphere through aquatic plants. *Environmental Quality*, (14): 40 - 46.
- Segers, R. (1998). Methane Production and Methane Consumption: A review of processes underlying wetland methane fluxes. *Biogeochemistry*, 41(1): 23-51.
- Sithole, A. (2009). Experimental methods for measuring the Q_{10} of methane and nitrous oxide emissions from rice agriculture. MS Thesis. Physics Department, Portland State University. 127p.
- Smith K.A., H. Clayton, I.P. McTaggart, P.E. Thomson, J.R.M. Arah, A. Scott, K.W.T. Goulding, J.L. Monteith, V.R. Phillips (1995). The measurement of nitrous oxide emissions from soil by using chambers. *Philosophical Transactions: Physical Sciences and Engineering*, 351(1696): 327-338.
- Smith, K.A. (1997). The potential for feedback effects induced by global warming on emissions of nitrous oxide by soil. *Global Change Biology*, 3: 327-338
- Smith, K.A., P.E. Thomson, H. Clayton, I.P. McTaggart and F. Conen (1998). Effects of temperature, water content and nitrogen fertilization on emission of nitrous oxide by soils. *Atmospheric Environment*, 32(19): 3301-3309.
- Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, O. Sirotenko, 2007: Agriculture. In *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, L.A. Meyer (eds)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Snover, A.K. and P.D. Quay (2000). Hydrogen and carbon kinetic isotope effects during soil uptake of atmospheric methane. *Global Biogeochemical Cycles*, 14: 25-39.

- Sugimoto, A. and E. Wada (1993). Carbon isotopic composition of bacterial methane in a soil incubation experiment: Contributions of acetate and CO₂/H₂, *Geochim. Cosmochim. Acta*, 57: 4015-4027.
- Stanford, G., S. Dzienia and R.A. Vander-Pol (1975). Effect of temperature on denitrification rate in soils. *Soil Science Society of America Journal*, 39(5): 867-870.
- Steinberg L.M. and Regan, J.M. (2009). mcrA-targeted real-time quantitative PCR method to examine methanogens communities. *Applied Environmental Microbiology*, 75(13): 4435-4442.
- Towprayoon S., K. Smakgahn, S. Poonkaew (2005). Mitigation of methane and nitrous oxide emissions from drained irrigated rice fields. *Chemosphere*, 59: 1547–1556.
- Turner, N.C. and Jarvis, P.G. (1975). Photosynthesis in Sitka spruce (*Picea sitchensis* (Bong.) Caw.) IV. Response to soil temperature. *Botany*, 12: 561-576.
- Tyler, S.C., R. S. Bilek, R.L. Sass and F.M. Fisher (1997). Methane oxidation and pathways of production in a Texas paddy field deduced from measurements of flux, δ¹³C, and δ²D of CH₄. *Global Biogeochemical Cycles*, 11(3): 323-348.
- V. Gauci, D. Fowler, S.J. Chapman and N.B. Dise (2004). Sulfate deposition and temperature controls on methane emission and sulfur forms in peat. *Biogeochemistry*, 71: 141–162.
- Valentine D.L., D.C. Blanton, W.S. Reeburgh, and M. Kastner (2001). Water column methane oxidation adjacent to an area of active hydrate dissociation. Eel River Basin. *Geochimica et Cosmochimica Acta*, 65(16): 2633-2640.
- van Hulzen, J.B., R. Segers, P.M. van Bodegom, P.A. Lefelaar (1999). Temperature effects on soil methane production: an explanation for observed variability. *Soil Biology and Biochemistry*, 31: 1919-1929.
- Wang B., H.U. Neue, H.P. Samonte (1997). The effect of controlled soil temperature on diel CH₄ emission variation. *Chemosphere*, 35(9): 2083-2092.
- Wang B., H.U. Neue, H.P. Samonte (1999). Factors controlling diel patterns of methane emission via rice. *Nutrient Cycling in Agroecosystems*, 53: 229–235.
- Wang B., Y. Xu, Z. Wang, Z. Li, Y. Ding, Y. Guo (1999). Methane production potentials of twenty-eight rice soils in China. *Biology and Fertility of Soils*, 29: 74–80.

- Wang, B., H.U. Neue, and H.P. Samonte (1999). Factors controlling diel patterns of methane emission via rice. *Nutrient Cycling in Agroecosystems*, 53: 229–235.
- Wang, Z.P., D. Zeng and W.H. Patrick Jr (1997). Characteristics of methane oxidation in a flooded rice soil profile. *Nutrient Cycling in Agroecosystems*, 49: 97-103.
- Wassmann, R., Lantin, R.S., Neue H-U. (eds) (2000). Methane emissions from major rice ecosystems in Asia. *Development in Plant and Soil Sciences* (91). Kluwer Academic Publishers, Dordrecht.
- Watanabe T., Kimura, M., Asakawa, S. (2009). Distinct members of a stable methanogenic archaeal community transcribe *mcrA* genes under flooded and drained conditions in Japanese paddy field soil. *Soil Biology and Biochemistry*, 41: 276-285.
- Watanabe, A., H. Yamada and M. Kimura (2005). Analysis of temperature effects on seasonal and interannual variation in CH₄ emission from rice-planted pots. *Agriculture, Ecosystems and Environment*, 105: 439-443.
- Whalen, S.C., and W.S. Reeburgh, 1996. Moisture and temperature sensitivity of CH₄ oxidation in boreal soils. *Soil Biological and Biochemistry*, 28: 1271-1281.
- Whiticar, M.J., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, 161 (1-3): 291-314.
- Whiting, G.J. and Chanton, J.P. (1993). Primary production control of methane emission from wetlands. *Nature*, 364: 794-795.
- Wilson, J.O., P.M. Crill, K.B. Bartlett, D.I. Sebacher, R.C. Harriss and R. Sass (1989). Seasonal variations of methane from a temperate swamp. *Biogeochemistry*, 8(1): 55 - 71.
- X. Chen, M.L. Cabrera, L. Zhang, J. Wu, Y. Shi, W.T. Yu and S.M. Shen (2002). Nitrous oxide emission from upland crops and crop-soil systems in northeastern China. *Nutrient Cycling in Agroecosystems*, 62: 241–247.
- Xu, S., P.R. Jaffé and D.L. Mauzerall (2007). A process-based model for methane emission from flooded rice paddy systems. *Ecological Modelling*, 205(3-4): 475-491
- Yang S-S., C-M. Liu c, C-M. Lai, Y-L. Liu (2003). Estimation of methane and nitrous oxide emission from paddy fields and uplands during 1990–2000 in Taiwan. *Chemosphere*, 52: 1295–1305.

- Yao H., and Z.L. Chen (1994a). Effect of chemical fertilizer on methane emission from rice paddies. *Geophysical Research*, 99(D8): 16 463-16470.
- Yao H., and Z.L. Chen (1994b). Seasonal variation of methane flux from Chinese rice paddy in semi arid, temperature region. *Geophysical Research*, 99(D8): 16471-16477.
- Yao H., R. Conrad, R. Wassmann and H.U. Neue (1999). Effect of soil characteristics on sequential reduction and methane production in sixteen rice paddy soils from China, the Philippines, and Italy. *Biogeochemistry*, 47: 269–295.
- Yao, H. and R. Conrad (1999). Thermodynamics of methane production in different rice paddy soils from China, the Philippines and Italy. *Soil Biology and Biochemistry*, 31: 463-473.
- Yu, Z.,X. Shangguan, D. Pollard and E.J. Barron. Simulating methane emission from a Chinese rice field as influenced by fertilizer and water level. *Hydrology Processes* (in press). DOI: 10.1002/hyp.1304
- Zhiyong, P., O. Hua, Z. Caiping, X. Xingliang (2009). Profile of methane concentrations in soil and atmosphere in Alpine steppe ecosystem on Tibetan Plateau. *Resources and Environment*, 7(1): DOI C92 X3
- Zou J., Y. Huang, L. Zong, X. Zheng, and Y. Wang (2004). Carbon dioxide, methane, and nitrous oxide emissions from a rice-wheat rotation as affected by crop residue incorporation and temperature. *Advances in Atmospheric Sciences*, 21(5): 691–698.

Appendix A

Seasonal average temperature data

Table A.1: Average soil temperature data measured for different treatments.

Date	WB1 (°C)	WB2 (°C)	WB3 (°C)	WB4 (°C)
10/30/2009	21.1	23.8	23.8	24.9
11/4/2009	21.2	23.4	28.0	32.1
11/5/2009	22.0	23.5	24.9	26.7
11/8/2009	20.7	23.9	27.4	32.5
11/12/2009	20.2	25.9	27.5	32.4
11/14/2009	20.3	24.8	27.9	31.8
11/18/2009	19.9	24.4	27.1	32.5
11/25/2009	20.2	24.5	27.4	33.0
11/30/2009	20.2	23.8	27.9	32.9
12/3/2009	19.6	23.4	28.0	32.2
12/3/2009	19.6	23.4	27.6	31.7
12/10/2009	19.4	24.2	25.7	28.8
12/26/2009	20.2	23.6	27.8	32.7
1/6/2010	20.0	23.5	26.9	30.9
1/13/2010	20.3	23.6	27.9	31.8
1/16/2010	20.4	23.6	28.0	30.9
1/20/2010	20.6	23.6	27.9	29.0
1/23/2010	20.2	23.4	27.9	32.4
1/27/2010	20.3	23.5	27.9	32.0
2/1/2010	20.2	23.4	27.9	31.8
2/3/2010	20.4	23.5	28.0	31.7
2/14/2010	19.5	20.6	26.6	32.0
2/14/2010	20.5	23.6	26.6	32.0
2/21/2010	20.4	22.9	27.7	32.0
3/10/2010	20.6	23.0	27.6	31.9
3/16/2010	20.4	22.9	27.6	31.3
3/24/2010	20.6	22.8	27.4	31.6
3/29/2010	21.2	23.1	27.9	32.1
3/31/2010	20.8	23.3	28.0	31.9
4/2/2010	20.2	23.0	27.9	32.5
4/7/2010	20.3	23.1	28.1	32.1

Appendix B

Average CH₄ and N₂O Flux Data

Table B.1: Average of average CH₄ fluxes (mg/m²/hr) from planted tubs from season 1

Date	WB2	WB1	WB3	WB4
10/25/2009	0.2	0.1	0.1	0.2
10/31/2009	0.3	0.2	0.7	1.6
11/5/2009	0.9	0.4	3.8	14.9
11/8/2009	1.5	0.4	5.4	13.6
11/12/2009	2.0	0.6	5.4	10.5
11/14/2009	2.4	0.6	6.1	15.7
11/25/2009	2.9	0.6	5.2	15.6
11/30/2009	2.6	0.6	7.4	12.1
12/3/2009	4.3	0.9	10.9	12.4
12/10/2009	8.8	1.4	14.5	27.8
12/16/2009	14.5	3.5	22.3	15.4
1/6/2010	22.9	10.0	28.9	19.1
1/11/2010	14.9	10.3	29.7	21.1
1/13/2010	24.7	11.5	27.9	18.4
1/16/2010	14.0	12.9	25.7	21.1
1/20/2010	14.0	11.4	27.7	29.3
1/23/2010	12.9	14.3	24.0	17.9
1/27/2010	15.7	12.1	25.9	19.5
2/1/2010	31.7	12.0	39.0	20.7
2/3/2010	19.3	12.5	34.7	19.9
2/10/2010	18.9	12.3	22.7	21.4
2/17/2010	13.5	12.6	15.4	24.7
2/21/2010	16.6	12.5	19.4	18.1
3/10/2010	22.7	14.3	46.3	27.6
3/16/2010	26.3	17.1	33.0	26.4
3/24/2010	29.8	17.0	34.4	24.2
3/29/2010	35.0	17.1	36.5	22.0
3/31/2010	29.4	20.7	32.0	23.5
4/2/2010	22.5	14.5	27.8	18.0
4/4/2010	21.0	11.5	30.8	17.1

Table B. 2: Average of average CH₄ fluxes (mg/m²/hr) from planted tubs from season 2

Date	WB2	WB1	WB3	WB4
4/24/2010	0.2	0.1	1.0	1.0
4/26/2010	0.4	0.1	1.7	3.7
5/1/2010	1.3	0.6	4.8	14.7
5/3/2010	1.5	0.8	6.3	24.3
5/10/2010	4.8	2.3	13.1	26.8
5/18/2010	9.9	5.8	24.3	29.3
6/2/2010	13.7	7.1	23.4	25.3
6/9/2010	12.8	8.3	22.1	27.0
6/17/2010	19.5	17.8	30.4	34.4
6/21/2010	25.1	21.6	33.8	39.7
6/24/2010	25.5	25.4	37.6	56.5
6/28/2010	31.2	28.9	44.8	53.7
6/30/2010	37.5	27.5	42.3	38.7
7/5/2010	30.7	26.9	36.6	43.4
7/6/2010	25.1	32.0	28.8	51.5
7/12/2010	37.4	37.0	40.2	51.1
7/19/2010	33.4	38.0	39.7	37.7
7/23/2010	34.6	35.8	33.3	34.2
7/28/2010	35.7	36.6	31.9	33.9
8/2/2010	19.5	29.4	37.0	25.1
8/9/2010	31.8	32.9	24.5	19.7
8/11/2010	17.9	17.4	36.8	16.6

Table B.3: Average of average N₂O fluxes (µg/m²/hr) from planted tubs from season 1.

Date	WB2	WB1	WB3	WB4
10/25/2009	62.5	47.3	-45.6	361.8
10/31/2009	509.2	302.8	683.5	881.5
11/5/2009	288.7	530.6	1167.3	600.6
11/8/2009	151.8	110.0	246.2	41.9
11/12/2009	21.0	61.2	53.7	35.0
11/14/2009	-1.5	21.5	20.4	14.8
11/25/2009	-9.7	218.9	40.0	-23.7
11/30/2009	-9.9	9.0	-6.2	30.5
12/3/2009	8.6	22.7	34.3	31.1
12/10/2009	11.6	31.6	46.8	25.2
12/16/2009	-0.3	19.4	-11.7	7.4
1/6/2010	25.7	30.7	16.3	-5.4
1/11/2010	17.6	22.6	31.1	54.3
1/13/2010	71.3	12.6	22.0	20.6
1/16/2010	11.8	-2.3	13.9	17.7
1/20/2010	-2.7	1.0	8.6	19.8
1/23/2010	-29.4	-	-	20.9
1/27/2010	-9.8	-15.0	-2.5	4.9
2/1/2010	3.0	3.4	26.5	54.1
2/3/2010	-8.1	23.0	28.3	-14.7
2/10/2010	4.6	1.0	-2.2	6.0
2/17/2010	-0.4	-4.7	24.0	92.3
2/21/2010	4.9	16.2	23.9	14.2
3/10/2010	21.0	12.5	-2.6	-10.9
3/16/2010	0.8	-1.0	11.8	20.1
3/24/2010	7.1	10.9	39.6	41.2
3/29/2010	20.0	36.4	-18.7	-25.6
3/31/2010	4.9	25.6	16.1	15.0
4/2/2010	-10.3	-12.9	-18.3	12.1
4/4/2010	18.6	11.8	12.8	14.7

Table B.4: Average of average N₂O fluxes (μg/m²/hr) from planted tubs from season 2.

Date	WB2	WB1	WB3	WB4
4/24/2010	822.5	146.4	702.4	-
4/26/2010	604.1	118.7	442.4	267.9
5/1/2010	53.8	38.7	82.8	38.3
5/3/2010	23.4	33.9	33.2	1.6
5/10/2010	21.1	19.0	9.4	40.1
5/18/2010	21.1	8.6	11.5	15.6
6/2/2010	12.6	-1.4	-11.7	15.2
6/9/2010	28.1	12.8	31.2	22.5
6/17/2010	15.3	-21.5	97.9	46.4
6/21/2010	5.7	8.3	1.9	34.9
6/24/2010	-6.6	8.3	27.1	34.9
6/28/2010	-6.6	-14.4	4.6	-27.3
6/30/2010	19.7	-3.4	1.9	8.2
7/5/2010	-24.2	-36.5	-1.8	17.2
7/6/2010	3.0	-15.1	-13.5	-6.4
7/12/2010	-2.9	15.8	23.2	7.1
7/19/2010	-37.9	-72.7	44.6	56.4
7/23/2010	11.1	-40.3	4.6	20.7
7/28/2010	8.4	-1.3	4.6	21.5
8/2/2010	-18.8	-20.4	-12.4	-6.5
8/9/2010	-27.1	-10.8	11.4	1.5
8/11/2010	-3.6	8.2	22.2	3.5
8/16/2010	41.6	3.4	-13.2	14.6

Appendix C

Average CH₄ and N₂O Production Data

Table C.1: Average CH₄ production data (mg/m²/hr)

Date	WB1	WB2	WB3	WB4
5/1/2010	51.3	23.3	84.4	6.8
5/4/2010	40.7	29.9	74.9	3.7
5/11/2010	108.9	100.1	162.3	506.7
6/15/2010	419.2	338.3	604.2	1592.9
6/25/2010	289.3	198.3	996.1	1269.5
7/2/2010	435.2	357.3	874.3	895.4
7/13/2010	694.0	186.0	727.2	2244.9
7/21/2010	1100.5	909.3	1567.4	1248.2
7/26/2010	763.6	715.5	2504.8	1350.3
7/29/2010	1787.8	1523.2	1349.2	2904.2
8/4/2010	*	*	2323.2	1869.0

Table C.2: Average N₂O production data (µg/m²/hr)

Date	WB1	WB2	WB3	WB4
5/1/2010	18.02	10.10	8.98	18.67
5/4/2010	103.21	55.55	8.09	10.19
5/11/2010	21.60	8.96	7.74	5.73
6/15/2010	6.44	6.66	8.78	6.29
6/25/2010	8.32	9.01	7.25	5.99
7/2/2010	10.48	6.20	7.75	25.51
7/13/2010	8.17	9.97	7.41	9.04
7/21/2010	6.85	7.09	8.16	8.40
7/26/2010	8.10	7.26	6.60	8.31
7/29/2010	8.26	8.37	9.66	7.78
8/4/2010	10.62	7.58	6.99	9.56

Appendix D

Pore Water CH₄ and N₂O concentrations

Table D.1: Average CH₄ pore water concentration (µg/L) data

Date	WB2	WB1	WB3	WB4
4/28/2010	72.6	41.7	141.7	804.8
5/4/2010	103.0	51.4	284.0	1609.2
5/18/2010	447.9	288.7	676.5	2198.3
6/2/2010	973.0	564.7	1399.4	1787.6
6/14/2010	2325.6	2160.1	4014.7	4754.3
6/18/2010	1835.1	2317.3	3175.0	3454.5
6/22/2010	2750.5	2633.3	3284.0	3922.9
6/29/2010	1359.9	2142.1	2984.7	2521.2
7/13/2010	3372.9	4081.1	5236.2	4328.5
7/19/2010	4617.6	5111.3	6135.3	6557.1
7/22/2010	5581.2	6525.4	7615.7	8899.0
7/26/2010	5971.7	6376.7	9580.7	9651.0
7/29/2010	7766.8	8318.9	7287.2	8256.2
8/3/2010	6193.9	6953.8	7554.1	7703.9
8/11/2010	7084.1	7111.3	9191.9	5197.3
8/18/2010	4911.1	3813.0	6254.4	4790.1
8/20/2010	5836.7	4041.4	4361.5	2259.0
Average	3372.9	3813.0	4361.5	4328.5
STDEV	2568.7	2703.9	3034.1	2737.5

Table D.2: Average N₂O pore water concentration (µg/L) data

Date	WB2	WB1	WB3	WB4
4/28/2010	438.7	1465.1	319.6	40.0
5/4/2010	255.6	1090.3	93.8	3.3
5/18/2010	6.4	209.0	0.8	0.7
6/2/2010	1.3	2.2	1.4	1.1
6/14/2010	0.6	0.7	0.6	0.6
6/18/2010	1.0	1.1	0.8	0.9
6/22/2010	0.3	0.7	0.3	0.4
6/29/2010	0.3	0.7	0.6	0.5
7/13/2010	1.4	1.3	1.1	1.2
7/19/2010	0.6	0.6	0.5	0.5
7/22/2010	0.6	0.9	1.0	0.9
7/26/2010	1.2	1.3	1.4	1.4
7/29/2010	1.0	1.1	1.1	1.0
8/3/2010	0.9	0.9	0.8	0.7
8/11/2010	0.9	1.4	0.9	0.9
8/18/2010	0.5	0.5	0.6	0.5
8/20/2010	0.8	1.7	0.8	1.1

Appendix E

Average CH₄ Isotope Measurements

Table E.1: Seasonal average $\delta^{13}\text{C}$ measured in flux samples from planted tubs (per mil)

DAT	WB1	WB2	WB3	WB4
9	-47.465	-49.485	-52.982	-56.905
16	-50.987	-52.835	-55.381	-52.000
23	-55.590	-57.749	-55.987	-55.135
31	-59.073	-58.926	-56.992	-56.202
46	-62.289	-62.428	-61.377	-60.006
53	-63.570	-60.029	-59.355	-59.241
65	-61.659	-61.619	-60.869	-59.711
72	-63.702	-61.240	-60.337	-56.632
80	-62.989	-60.977	-59.948	-57.507
86	-62.280	-60.751	-57.658	-56.285

Table E.2: Seasonal average $\delta^{13}\text{C}$ measurements in flux samples from unplanted tubs

Date	WB1	WB2	WB3	WB4
9	-46.083	-49.63	-48.859	-53.771
16	-47.403	-49.127	-50.481	-48.616
23	-50.355	-52.921	-47.705	-48.018
31	-51.650	-51.988	-49.618	-52.018
46	-54.595	-50.692	-52.962	-54.290
53	-50.587	-55.407	-53.963	-55.472
65	-63.796	-55.677	-52.366	-51.454
72	-54.858	-53.932	-54.273	*
80	-49.272	-57.281	-56.295	-55.826
86	-46.083	-56.437	-56.334	-57.060

Table E.3: Seasonal average δD values from flux samples (per mil)

δD in fluxes from planted tubs				δD in fluxes from unplanted tubs			
WB1	WB2	WB3	WB4	WB1	WB2	WB3	WB4
-146.046	-166.382	-209.546	-287.525	-151.878	-174.952	-172.768	-277.643
-147.235	-212.793	-280.145	-306.022	-87.427	-172.137	-218.847	-322.953
-234.46	-268.766	-304.434	-337.628	-143.203	-284.005	-186.047	-308.278
-287.884	-312.999	-284.767	-342.698	-199.287	-304.213	-260.519	-344.032
-309.998	-320.447	-333.797	-342.777	-251.886	-264.365	-282.675	-338.05
-317.715	-341.855	-340.58	-339.573	-311.462	-341.453	-330.48	-347.87
-329.468	-334.977	-342.555	-347.007	-229.64	-343.223	-273.266	-248.686
-350.217	-347.207	-351.853	-327.494	-350.081	-290.649	-311.98	-336.047
-340.916	-350.789	-349.817	-339.8	-324.291	-309.332	-326.923	-340.26
-331.616	-354.371	-347.782	-352.106	-298.5	-328.014	-341.866	-344.473

Table E.4: Seasonal average $\delta^{13}C$ values from pore water samples (per mil)

Average $\delta^{13}C$ values in pore water from planted tubs				Average $\delta^{13}C$ values in pore water from unplanted tubs			
WB1	WB2	WB3	WB4	WB1	WB2	WB3	WB4
-36.392	-41.110	-35.319	-49.114	-27.11	-44.455	-50.815	-56.367
-51.316	-52.760	-55.491	-45.118	-55.18	-55.848	-53.738	-46.481
-51.974	-51.694	-41.605	-44.779	-46.22	-51.940	-45.958	-47.207
-50.427	-35.569	-47.980	-48.529	-52.57	-50.942	-47.145	-51.413
-54.740	-51.377	-48.297	-50.250	-54.08	-52.595	-52.576	-52.313
-55.080	-51.097	-52.191	-52.206	-53.72	-54.089	-54.406	-48.528
-52.004	-47.997	-49.765	-47.882	-52.18	-52.733	-52.992	-53.229
-44.280	-17.157	-42.422	-39.603	-42.98	-43.289	-49.154	-46.342
-45.652	-33.528	-43.343	-47.520	-45.83	-50.229	-50.069	-48.156
-51.983	-50.577	-51.660	-52.072	-56.53	-56.845	-55.899	-55.579

Table E.5: Seasonal average δD values from pore water measurements (per mil)

<i>δD Averages in pore water from planted tubs</i>				<i>δD averages in pore water from unplanted tubs</i>			
WB1	WB2	WB3	WB4	WB1	WB2	WB3	WB4
*	*	*	-254.92	*	*	-164.5	-240.8
*	-250.695	-330.13	-305.918	*	-277.35	-300.7	-371.17
-218.503	-248.073	-300.608	-331.81	*	-248.154	-295.6	-312.57
-261.415	-273.68	-271.195	-298.53	-256.03	*	-358.7	-340.065
-356.315	-367.688	-261.105	-331.518	-360.645	-348.105	-317.9	-321.955
-358.198	-361.465	-365.31	-367.35	-348.73	-330.675	*	*
-222.476	-201.412	-162.474	-152.959	-215.853	-202.823	-162.2	-175.161
-297.353	-203.298	-306.89	-284.405	-208.8	-231.513	-357.0	-317.475
-298.335	-242.815	-310.218	-323.145	-298.175	-311.925	-315.4	-322.775
-367.288	-380.133	-337.405	-359.38	-321.757	-377.605	-358.8	-350.72