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Feedbacks of Methane and Nitrous Oxide Emissions from Rice Agriculture

Alec Sithole
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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:
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Abstract

The effect of global warming on methane (CH$_4$) and nitrous oxide (N$_2$O) 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 CH$_4$ and N$_2$O emissions were determined and calculated as the Q$_{10}$s 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 CH$_4$ 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$^\circ$C, above which emissions decreased with increasing temperature. The seasonal average Q$_{10}$s of CH$_4$ 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 $^\circ$C. Considering that the processes of CH$_4$ production and emission are similar to those in natural wetlands, which is the largest source of atmospheric CH$_4$, the contribution of this feedback is likely to cause a significant increase to the present CH$_4$ atmospheric budget if the current global warming trend persists over the next century.
The $Q_{10}$ 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.

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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 ± 0.05 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).
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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).
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 CH$_4$ emission from rice paddies are production, oxidation and transport. Modified diagram adapted from Neue and Roger (1993).

Another important greenhouse gas, N$_2$O, is about 300 times more effective in global warming than CO$_2$ 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), N$_2$O has a GWP of 2.8 Gt CO$_2$- 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 CH$_4$ and N$_2$O 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 CH$_4$ and N$_2$O 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 Q$_{10}$ of the net emissions? (II) What is the linkage between the Q$_{10}$ 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 CH$_4$ and N$_2$O 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 $Q_{10}$ of CH$_4$ and N$_2$O

Already, several studies have shown that CH$_4$ and N$_2$O fluxes increase with increasing soil temperature (Table 1.1). The large variation in the reported $Q_{10}$ 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 $Q_{10}$ 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.
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<td>Khalil et al., 1998d</td>
</tr>
<tr>
<td>&lt;1 – 6</td>
<td>CH$_4$ emissions from flooded soils</td>
<td>Incubation flooded soil samples</td>
<td>15 – 25</td>
<td>Rath et al., 2002</td>
</tr>
<tr>
<td>&gt;1- 5</td>
<td>CH$_4$ emissions from flooded soils</td>
<td>Incubation flooded soil samples</td>
<td>25 - 35</td>
<td>Tsutsuki and Ponnampuruma, 1987</td>
</tr>
<tr>
<td>16</td>
<td>CH$_4$ emissions from flooded soils</td>
<td>Incubated paddy soil cores</td>
<td>20 - 25</td>
<td>Tsutsuki and Ponnampuruma, 1987</td>
</tr>
<tr>
<td>6</td>
<td>N$_2$O emission</td>
<td>Automated flux chamber measurements from potato field soils.</td>
<td>10 - 30</td>
<td>Flessa et al., 2002</td>
</tr>
<tr>
<td>50</td>
<td>N$_2$O emission</td>
<td>Incubated soil cores fertilized with ammonium nitrate.</td>
<td>5 - 50</td>
<td>Dobbie and Smith 2001</td>
</tr>
<tr>
<td>8.9</td>
<td>N$_2$O emission</td>
<td>Incubated soil cores fertilized with ammonium nitrate.</td>
<td>12 - 18</td>
<td>Dobbie and Smith 2001</td>
</tr>
<tr>
<td>3.7</td>
<td>CH$_4$ production</td>
<td>Incubated grassland soil cores</td>
<td>5 - 50</td>
<td></td>
</tr>
<tr>
<td>2.3</td>
<td>CH$_4$ production</td>
<td>Incubated grassland soil cores</td>
<td>12 - 18</td>
<td></td>
</tr>
<tr>
<td>2.2-7.7</td>
<td>CH$_4$ production</td>
<td>Emission from rice ecosystems in the greenhouse</td>
<td>19 -23</td>
<td>Sithole, 2009</td>
</tr>
<tr>
<td>1.1-3.6</td>
<td>N$_2$O</td>
<td>Incubated soil cores</td>
<td>5 - 50</td>
<td>Dobbie and Smith 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incubated soil cores fertilized with ammonium nitrate.</td>
<td>12 - 18</td>
<td>Dobbie and Smith 2001</td>
</tr>
</tbody>
</table>

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$_2$O were carried out. From this scoping study, $Q_{10}$s for CH$_4$ and N$_2$O were found 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 mg/m$^2$/hr for seasonal averages to 50 mg/m$^2$/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 Q10s cannot be extrapolated either. Instead the factors that control the Q10 are needed for extrapolation and upscaling. In the quest for answers to these questions, the feedbacks between global warming and two non-CO2 greenhouse gases, CH4 and N2O, 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 CH4 emission, production and oxidation from a mechanistic approach. The same experiments were used to investigate behavior of N2O emissions and production under different temperature treatments under greenhouse conditions.

1.4 Scope and General Outline

In this work, the Q10s were determined from a mechanistic point of view. Experiments were designed, constructed and tested as outlined in Chapter 2. The Q10s 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 CH4 in pore water were investigated and results are presented in Chapter 6. Considering that CH4 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$_2$O 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) tubs 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 CH$_4$ and N$_2$O 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 ThermoSheath 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 Therasheath 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.
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$_2$O 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 CH₄ and N₂O 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 CH$_4$ leakage. After a known dose of 1 % CH$_4$ 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 CH$_4$ and N$_2$O 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) CH$_4$, and (b) N$_2$O. $H_0$ was rejected for $t_{a=0.025} \leq -3.184$ and $t_{a=0.025} \geq +3.184$ for $n = 5$ (two-tailed test).

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

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 CH$_4$ and N$_2$O 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 CH$_4$ 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 CH$_4$ 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 CH$_4$ 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 CH$_4$ concentrations in future.

3.1 Introduction

The current global CH$_4$ emission rate from natural and agricultural sources is about 40-50 % of the total CH$_4$ emissions (Whiting and Chanton 1993). Irrigated rice agriculture contributes an estimated 10-15 % of the total global CH$_4$ 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 CH$_4$ emissions from the root zone to the atmosphere. Several studies have indicated that methane fluxes increase when the temperature was elevated (Holzapfel-Pschor 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-Pschor 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, CH$_4$ 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 CH$_4$ fluxes in heating experiments, and (II) the magnitude of this feedback, are not yet known.

The relationship between soil temperature and CH$_4$ 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 ($8.31 \text{ Jmol}^{-1}\text{K}^{-1}$).
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 ºC. 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 ºC (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). Parashar et al. (1993) reported a distinct increase in CH$_4$ emission from rice plots with increase in soil temperature from 26 to 34.5 ºC 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 ºC.
Similar results were observed in the previous greenhouse experiments (Sithole, 2009) and in plots of CH$_4$ flux versus temperature shown in Pangala et al. (2010). Because of the large variation in Q$_{10}$ values reported from various studies, the major challenge is the unification of these results. This limits the accuracy of model estimates of future global CH$_4$ emissions under a warmer world. To improve the quantification of this temperature feedback and model output, knowledge of the representative Q$_{10}$ 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 CH$_4$ fluxes and to determine how CH$_4$ 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 CH$_4$ 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).
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 though the septum into four sampling columns of the GC, with nitrogen as the carrier gas. The net CH$_4$ fluxes $F$ [in $\mu$g m$^{-2}$ 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):

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

Where \( V_{\text{chamber}} \), \( V_{\text{correction}} \) and \( \gamma \) 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. \( A_{\text{surface}} \) 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 \( \alpha \) 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:

\[
Q_{10} = \frac{\int Q_{10}(t) F(t) \, dt}{\int F(t) \, dt}
\]

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 CH$_4$ fluxes were observed. In planted tubs under different temperature treatments, CH$_4$ fluxes increased from zero at the start of the growing period, reaching maxima of between 20 and 60 mg/m$^2$/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/m$^2$/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 CH$_4$ transport from the soil to the atmosphere. The other factor is an increase in dissolved CH$_4$ pore water concentration.

This study also indicated that CH$_4$ fluxes were positively correlated with soil temperature at 10 cm depth, and that temperature was major driving factor in the increase of CH$_4$ fluxes, consistent with reports from other studies (Schütz et al., 1989). CH$_4$ fluxes differed greatly between different temperature treatments. Lowest CH$_4$ fluxes were observed at the lowest temperature set-up (20 °C), whist 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 CH₄ fluxes for growing seasons.

Season 1

Season 2
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.](image)
Segers (1998) proposed that the observed high $Q_{\text{10}}$ 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 $Q_{\text{10}}$ values. Hence these values are not a true reflection of the response between CH$_4$ fluxes from rice ecosystems. These high $Q_{\text{10}}$ 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: $Q_{\text{10}}$ of CH$_4$ fluxes during the growing season, showing high values of $Q_{\text{10}}$ for low flux values.](image)

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 $Q_{\text{10}}$
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.
4.0 Summary

Concentrations of dissolved CH$_4$ 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 CH$_4$ is emitted to the atmosphere. The concentrations of CH$_4$ 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 CH$_4$ 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 CH$_4$ concentrations on temperature was more distinct than in any other part of the season. When CH$_4$ fluxes were compared to pore water concentrations for > 60 DAT, the fluxes flattened out around 30 mg/m$^2$/hr despite increases in pore water concentrations. From this observation, we inferred that CH$_4$ 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 CH$_4$ 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 CH$_4$ concentration in pore water, among other environmental factors. 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 CH$_4$ emissions from rice paddies.

Increases in soil temperatures due to global warming may cause significant changes to the rates of fundamental processes that produce CH$_4$ in rice paddies. To understand these impacts, it is important to make an assessment of the CH$_4$ 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 CH$_4$ in rice paddies, and (III) to evaluate CH$_4$ concentrations in rice paddy soil. The objective was to understand how an increase in temperature would affect the CH$_4$ pool from which CH$_4$ 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.](image)

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):

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

Where \([X]_h\) and \([X]_a\) are the headspace and the ambient air concentrations (in \(\mu\text{molL}^{-1}\)); \(V_h\) 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), \(\rho_x\) is the density of the gas, respectively.
4.4 Results and Discussion

4.4.1 Profiles of dissolved CH$_4$ in the rhizosphere

Results indicated that from 0 to 15 cm, there was a general increase in the concentration of dissolved CH$_4$, with slight differences between different temperature treatments (Fig. 4.3). Similarly, Rothfuss and Conrad (1993) reported CH$_4$ 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 CH$_4$ 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 CH$_4$ 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 CH$_4$ 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 CH$_4$ oxidizing-bacteria than other layers. Thus, as the season progressed,
the number of CH$_4$ 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, CH$_4$ concentrations were much higher in the 10-15cm layer. This dependence on temperature could also be due to stimulation of bacterial population growth (Holzapfel-Pschorr et al., 1985; Dunfield et al., 1993) or enzyme synthesis that results in the production of CH$_4$ (Schutz et al., 1990), or a combination of both. High seasonal fluctuations were also common in the near-surface zone (0-5 cm).

![Graph showing temperature effect on CH$_4$ concentration](image)

**Fig. 4.3**: Temperature effect on the seasonal average CH$_4$ 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 CH$_4$ 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 CH$_4$ 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 CH$_4$ 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 CH$_4$ 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...
CH$_4$ concentration profiles reported by Rothfuss and Conrad (1993). This transition could be due to CH$_4$ accumulation under limited transport and oxidation processes.

In all treatments, differences of CH$_4$ 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 CH$_4$ 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 CH$_4$ 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 CH$_4$ 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 O$_2$ 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 CH$_4$ 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 CH$_4$ 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 CH$_4$ and its accumulation was more governed by availability and population size of both CH$_4$ 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 O$_2$ into the soil and CH$_4$ into the atmosphere (Gross et al., 1993).

4.4.2 Temporal Variations

Results showed that in all treatments, dissolved CH$_4$ 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 CH$_4$ concentration and soil temperature at all depths during the first half of the season, with stratification between different layers. In general, the correlations between CH$_4$ concentration and soil temperature at different depths were weak. Seasonal patterns of CH$_4$ fluxes closely followed the same dynamics of CH$_4$ concentrations between 0 and 60 DAT.
Fig. 4.5: Effect of temperature on CH$_4$ concentration in pore water, showing higher concentrations at 32 $^\circ$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 µg/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 CH₄ 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.

![Graph](image)

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.
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 CH$_4$ concentrations across different temperatures. Concentration of CH$_4$ 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 CH$_4$ concentrations were associated with higher temperatures (Fig. 4.7). This was an indication that the temperature effect on the relationship between pore water CH$_4$ concentration and fluxes seem to exist between 0 and 60 DAT. In one observation (5cm, 32 °C), average pore water CH$_4$ 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 CH$_4$ concentration was the limiting factor in CH$_4$ fluxes. During this period fluxes increased to a maximum. In all treatments, CH$_4$ concentrations were highest and lowest at 32 °C and 20 °C.
Emission rates from rice fields are correlated to CH$_4$ concentration in the pore water during the first half of the season. Between 1 and DAT 60, pore water CH$_4$ concentration (Fig. 4.9) was the limiting factor in CH$_4$ fluxes (Fig. 4.10). This limiting point could be an indication that fluxes remain almost constant even though CH$_4$ pool increased in the pore water.

Fig. 4.11: CH$_4$ fluxes reaches maximum (25-40 mg/m$^2$/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 CH$_4$ 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 CH$_4$ concentration accumulation. This implies that although the process that produces CH$_4$ is temperature-dependent, this process is limited by other factors. One possible factor may be that as the growing season progresses, the O$_2$ 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 CH$_4$ can diffuse into the root system.

4.5 Conclusion
Pore water CH$_4$ 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 CH$_4$ in the floodwater. The results also suggest that once the CH$_4$ 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 CH$_4$ to the atmosphere. On the other hand, these results also indicate that the CH$_4$ 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 CH$_4$ 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 CH$_4$ production was highly dependent on soil temperature over the season. The calculated seasonal Q$_{10}$ of CH$_4$ 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 Q$_{10}$ 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 CH$_4$, and consequently emissions if oxidation does not increase by the same factor. Considering that the processes involved in the production of CH$_4$ in rice ecosystems is similar to that in natural wetlands, the atmospheric CH$_4$ contribution due to global warming feedback is likely to change the current total biotic CH$_4$ contribution.

5.1 Introduction
The most fundamental process in the emission of CH$_4$ from rice agriculture is the production process. However, the rate of CH$_4$ emissions from these ecosystems is a
composite of production, transport and oxidation processes. In rice paddies, \( \text{CH}_4 \) 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 \( \text{CH}_4 \). These methanogenic bacteria utilize substrates such as \( \text{H}_2/\text{CO}_2 \), formate, methanol, methylamines, acetate for growth and \( \text{CH}_4 \) 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 \( \text{Fe(III)} \) and sulfate (Watanabe, 1984), and many other environmental factors such as temperature (Tsutsuki and Ponnampерума 1987). The most important factors that control \( \text{CH}_4 \) 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 \( \text{CH}_4 \) in flooded rice soils are controlled by many soil, plant, and microbial factors. \( \text{CH}_4 \) production rates of soils are significantly and linearly correlated with soil organic matter content (Wang et al., 1999). The extent and duration for which \( \text{CH}_4 \) 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 \( \text{CH}_4 \) in the root zone by providing methanogenic substrates through root exudates, decaying root tissues, and by respectively transporting \( \text{O}_2 \) and \( \text{CH}_4 \) 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).

![Graph showing the effect of temperature on the percentage contribution of acetate and H$_2$/CO$_2$ to methanogenesis in anoxic rice soil.](image)

**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$_2$O 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 Ponnampерума, 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 N₂ 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), CH$_4$ 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 CH$_4$ is thought to be a characteristic of the soil redox potential, pH, substrate availability, and temperature. For example the production of CH$_4$ 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 CH$_4$ production: root oxygen transport suppresses CH$_4$ production, whereas root decay and root exudation promote CH$_4$ production (Sass et al., 1991; Segers, 1998).
Fig. 5.3: CH$_4$ 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 CH$_4$ 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 Fe$^{3+}$ and SO$_4^{2-}$ ions (van Hulzen et al., 1999) so that the beginning of CH$_4$ 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 CH$_4$ concentrations in rice tubs, but had no net effect on the CH$_4$ 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 CH$_4$ production. Similarly,
Wang et al. (1999) found that CH$_4$ 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, CH$_4$ is predominantly produced from H$_2$/CO$_2$, 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 CH$_4$ 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 CH$_4$ 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 CH$_4$ Production

The Q$_{10}$ of CH$_4$ production did not change with increasing DAT, varying from <1 and 8.5 (Fig. 5.4).
Fig. 5.4: (a) Average CH$_4$ production rates as a function of temperature, and (b) Q$_{10}$ values of CH$_4$ 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 mg/m$^2$/hr to about 230 mg/m$^2$/hr, following a temperature increase of $20 - 32 \, ^\circ C$. 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).
5.4.4 Estimation of $Q_{10}$ of Transport

Based on the $Q_{10}$s production, flux and oxidation, the $Q_{10}$ 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 $Q_{10}$ 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 CH$_4$ production under continuously flooded rice ecosystems was successfully determined in this research. The rate of CH$_4$ production was strongly dependent on soil temperature, with a seasonally averaged $Q_{10}$ 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 $\text{CH}_4$ 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 CH$_4$ 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. CH$_4$ fluxes, below- and aboveground carbon ($\delta^{13}$C) and hydrogen ($\delta^D$) isotopic composition of CH$_4$ 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 CH$_4$ was extracted into N$_2$ headspace. Isotopic measurements showed that the mean aboveground $\delta^{13}$C values ranged between -60.4 and -53.0 ‰, with an average of -56.7 ‰ relative to V-PDB, while $\delta^D$ 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 $\delta^{13}$C and $\delta^D$, respectively. CH$_4$ flux ranged from near zero to 30-60 mg/m$^2$/hr in mid-season corresponding to a rise in pore water CH$_4$ to 8-12 mg/L. These results were integrated and interpreted using an empirically-driven concentration and isotope model to understand CH$_4$ dynamics and to examine the effect of temperature on mechanisms that control CH$_4$ emissions. The $Q_{10}$ of methane oxidation was found to be 1.2 and 1.4-2.4 from the $\delta^{13}$C and $\delta^D$ values, respectively. This study showed that temperature plays a less important role in CH$_4$ oxidation in rice.
paddies compared with production (reported in Chapters 3 and 5) where the average Q10s were 4.6 and 3.1, respectively. Thus the change in the CH4 oxidation efficiency due to elevated soil temperature would not be enough to offset the effect of temperature on CH4 production and emissions, thereby increasing the likelihood of increased CH4 emissions from rice agriculture under future global warming.

6.1 Introduction

Rice paddies represent a sizable anthropogenic source of CH4 emissions into the atmosphere. Rhizospheric oxidation is a major sink of CH4 in rice ecosystems. Without this process, CH4 emissions from rice agriculture would be perhaps an order of magnitude higher than the present rates. Yet the impact of global warming on CH4 oxidation, and on the global CH4 atmospheric budget remains poorly understood. Globally, CH4 oxidation is a sink for CH4 and is important in reducing the CH4 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 CH4 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 CH4 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 CH4 oxidation is considered a key determinant of the rate of change of atmospheric CH4 concentration (Wang and Ineson, 2003).
It is estimated that up to 90% of the CH$_4$ 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 CH$_4$ 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 O$_2$ from the atmosphere that diffuses into the soil or the root zone through plants to oxidize CH$_4$. An aerobic surface layer develops around the plant roots. The thickness of this layer is determined by the net consumption and transport rates of O$_2$ 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 O$_2$ supply to the rhizosphere is a decisive factor in the magnitude of CH$_4$ emission. Hence, rhizospheric CH$_4$ oxidation is an important internal sink for CH$_4$ produced in the flooded soils, consuming up to 90% of CH$_4$ production. The presence of O$_2$ in the rhizosphere limits CH$_4$ production and enhances oxidation (Wang et al., 1997). This supply of O$_2$ to the rhizosphere enhances the activity of aerobic methanogens leading to higher CH$_4$ oxidation efficiency. Thus, under these conditions, both plant-mediated CH$_4$ emission and the percentage of CH$_4$ oxidized increase. It is thought that approximately 580 Tg y$^{-1}$ of CH$_4$ 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 CH$_4$ budget.
6.1.1 Other Factors Affecting CH$_4$ Oxidation in Flooded Soils

The elements influencing CH$_4$ oxidation in flooded soils depend on factors that affect methanotrophic activities such as O$_2$, temperature, CH$_4$ availability and the mode of CH$_4$ transport from the production zone to the atmosphere. It is estimated that the optimum temperature for CH$_4$ oxidation ranges between 25 and 30 °C (Whalen and Reeburgh, 1996). In the same studies, CH$_4$ 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 $\delta^{13}$C values by 1-2 ‰ than plants with nitrogen (N) or potassium deficiency (O’Leary, 1981). The kinetics of CH$_4$ 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 CH$_4$. Several studies have demonstrated that elevated soil N, particularly in the form of NH$_4^+$, may reduce CH$_4$ oxidation rates. In particular, increased inhibition of CH$_4$ oxidation by NO$_3^-$ has been observed at low CH$_4$ 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 CH$_4$ from the production (anaerobic) zone and the transport of O$_2$ from the atmosphere into the soil (Hanson and Hanson, 1996). In planted flooded soils, O$_2$ is transported into the root zone via the plants. Another important factor may be the
consumption of O\textsubscript{2} 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 CH\textsubscript{4} oxidation

Several approaches have been used to quantify CH\textsubscript{4} oxidation in the rhizosphere: (I) mass balance calculations based on known concentration of CH\textsubscript{4} (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 N\textsubscript{2}, and (V) measurement of 13C-isotopic signatures of the produced CH\textsubscript{4} and CO\textsubscript{2} (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 CH\textsubscript{4} and CO\textsubscript{2} emission and production measurements. The rate of CH\textsubscript{4} 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 CH$_4$ is a direct and non-invasive technique used to monitor the CH$_4$ cycle processes at a finer scale than is possible by measurement of surface CH$_4$ emissions only (Whiticar et al., 1986). The technique has found extensive use in the assessment of biogeochemical processes such as CH$_4$ production and oxidation in landfills, terrestrial and marine environments (Nakagawa et al., 2002). The method is based on determining the proportion (fraction) of CH$_4$ oxidized from the differences between stable and non-destructive hydrogen and carbon isotopic ratios. The technique is widely used to quantify CH$_4$ 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 CH$_4$ has a carbon isotopic composition below -75 ‰ (Tab. 6.1), but due to oxidation, CH$_4$ may exhibit $^{13}$C enriched values of -30 to -50 ‰. These methanotrophic bacteria preferentially consume $^{12}$CH$_4$ and discriminate against the heavier $^{13}$CH$_4$ (Chanton and Liptay, 2000; De Visscher et al., 2004; Happell et al., 1994; Whiticar 1999) leaving residual CH$_4$ enriched in $^{13}$CH$_4$ (O’Leary et al., 1988; Reeburgh et al., 1993). The resultant effect is a change in the isotope composition when CH$_4$ is oxidized, thereby altering the isotope ratio. Because of this property, CH$_4$
oxidation is estimated by the degree of change between the $\delta^{13}C$ content of CH$_4$ emitted relative to the $\delta^{13}C$ of CH$_4$ in the anoxic zone.
Table 6.1. Overview of typical isotope measurements on CH₄ from rice paddies.*

<table>
<thead>
<tr>
<th>Location</th>
<th>δ¹³C</th>
<th>δD</th>
<th>Seasonal Coverage</th>
<th>Sampling Method/Sample Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>-68 to -66</td>
<td>⋮</td>
<td>3 weeks before harvest</td>
<td>nocturnal inversion</td>
<td></td>
</tr>
<tr>
<td>Kenya</td>
<td>-63 to -57</td>
<td>⋮</td>
<td>days 44, 72, 100, 128, 156 (after sowing)</td>
<td>static chamber</td>
<td>[Tyler et al., 1988]</td>
</tr>
<tr>
<td>Louisiana</td>
<td>-66 to -60</td>
<td>⋮</td>
<td>May–June</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>-68 to -63</td>
<td>-339 to -319</td>
<td>days 85–88 (after flooding)</td>
<td>static chamber</td>
<td>[Wahlen et al., 1989]</td>
</tr>
<tr>
<td>Japan</td>
<td>-68 to -61</td>
<td>⋮</td>
<td>throughout the season (maximum interval 17 days)</td>
<td>gas bubbles</td>
<td>[Usaki et al., 1991]</td>
</tr>
<tr>
<td></td>
<td>-65 to -61</td>
<td>-310 to -290</td>
<td>⋮</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-71 to -56</td>
<td>⋮</td>
<td>days 22, 51, 71, 93, 107 (1990)</td>
<td>static chamber</td>
<td>[Wahlen, 1993]</td>
</tr>
<tr>
<td></td>
<td>-72 to -58</td>
<td>⋮</td>
<td>days 38, 72, 100 (1991) (after flooding)</td>
<td></td>
<td>[Tyler et al., 1994]</td>
</tr>
<tr>
<td>Louisiana</td>
<td>-66 to -61</td>
<td>⋮</td>
<td>days 84–89 (after seeding)</td>
<td>CO₂ controlled chamber</td>
<td>[Chanon et al., 1997]</td>
</tr>
<tr>
<td></td>
<td>-61 to -57</td>
<td>-325 to -300</td>
<td>⋮</td>
<td>gas bubbles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-56 to -50</td>
<td>⋮</td>
<td></td>
<td>lacunal</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>-71 to -52</td>
<td>-352 to -311</td>
<td>every 7 days</td>
<td>static chamber</td>
<td>[Bergamaschi, 1997]</td>
</tr>
<tr>
<td></td>
<td>-71 to -58</td>
<td>-358 to -343</td>
<td>every 1–4 weeks</td>
<td>gas bubbles</td>
<td></td>
</tr>
</tbody>
</table>

*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):

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

Where \( R_{\text{sample}} \) is the carbon-13 abundance ratio given by \(^{13}\text{CH}_4/^{12}\text{CH}_4\) ratio of the sample.

Since absolute values of \(^{13}\text{C}/^{12}\text{C}\) are difficult to obtain, the values are reported relative to Vienna Peedee Belemnite (VPDB) (O’Leary, 1981; Gonfiantini et al., 1995). \( R_{\text{standard}} \) is the \(^{13}\text{CH}_4/^{12}\text{CH}_4\) ratio for V-PDB. Negative δ\(^{13}\text{C}\) values indicate that depletion of the \(^{13}\text{C}\) 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 \( R_{\text{standard}} \) (Gonfiantini et al., 1995). The percentage fraction of oxidized CH₄ (oxidation efficiency) is determined as a function of isotopic belowground CH₄ (δ₁), emitted CH₄ (δₑ), and the kinetic isotope effect (KIE) during oxidation (α):
\[ F = \frac{\delta_i - \delta_f}{(\alpha - 1)(\delta_f + 1000)} \]

Where \( \delta_i \) is the \( \delta^{13}C \) (or \( \delta^D \)) value in the anoxic zone, \( \delta_f \) is the \( \delta^{13}C \) (or \( \delta^D \)) value of emitted \( CH_4 \), \( \alpha \) is the fractionation factor, \( \alpha \) for microbial oxidation and \( \alpha_{\text{trans}} \) is the fractionation factor associated with gas transport. The extent of bacterial oxidation is estimated by \( \alpha \), which is the difference between the production and the residual \( CH_4 \). Typical values of \( \alpha \) associated with \( \delta^{13}C \) and \( \delta^D \) values are presented in Table 6.2.
<table>
<thead>
<tr>
<th>Study</th>
<th>$\alpha_{ox}$</th>
<th>$\alpha_{cox}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory enrichment cultures of methanotrophic bacteria</td>
<td>---</td>
<td>1.011</td>
<td>Silverman and Oyama [1968]</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>1.016</td>
<td>Lebedew et al. [1969]</td>
</tr>
<tr>
<td></td>
<td>1.103 - 1.311</td>
<td>1.0132 - 1.025</td>
<td>Zyakun et al. [1988]</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>1.0052 - 1.0313</td>
<td>Coleman et al. [1981]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Barker and Fritz [1981]</td>
</tr>
<tr>
<td>Landfill cover soils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>1.044 ± 0.020</td>
<td>1.008 ± 0.003</td>
<td>Bergamaschi and Harris [1995]</td>
</tr>
<tr>
<td>Europe</td>
<td>1.039 ± 0.026</td>
<td>1.008 ± 0.004</td>
<td>Bergamaschi et al. [1998]</td>
</tr>
<tr>
<td>NE United States</td>
<td>1.046 ± 0.016</td>
<td>1.022 ± 0.008</td>
<td>Liptay et al. [1998]</td>
</tr>
<tr>
<td>Swamp floodwaters</td>
<td>1.050 - 1.129</td>
<td>1.003 - 1.021</td>
<td>Happell et al. [1994]</td>
</tr>
<tr>
<td>Soils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boreal forest soils in situ (Alaska)</td>
<td>---</td>
<td>1.022 - 1.025</td>
<td>Reeburgh et al. [1997]</td>
</tr>
<tr>
<td>Native grassland soils (Washington)</td>
<td>1.136 - 1.209</td>
<td>1.0121 - 1.0183</td>
<td>Snover and Quay [2000]</td>
</tr>
<tr>
<td>Temperate forest soils (Washington)</td>
<td>1.094 - 1.128</td>
<td>1.0165 - 1.0177</td>
<td>Snover and Quay [2000]</td>
</tr>
</tbody>
</table>

*Adapted from Snover and Quay [2000]
Typically, values of $\alpha_{ox}$ 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 $\alpha_{ox}$ and soil temperature (Fig. 6.1).

![Graph showing typical models for the variation of $\alpha_{ox}$ with temperature.](image)

**Fig. 6.1:** Typical models proposed for the variation of $\alpha_{ox}$ 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 CH$_4$ 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, CH$_4$ 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 CH$_4$ 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 $^{12}$CH$_4$ relative to $^{13}$CH$_4$ resulting in the enrichment of $^{13}$CH$_4$ 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 CH$_4$ (Chanton, 2004), the main transport mechanism at night is diffusion (passive transport), which result in mass-dependent fractionation of emitted CH$_4$ (Chanton and Whiting, 1996). The presence of diffusion results in the release of depleted CH$_4$ 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 \( \text{CH}_4 \), resulting in a reduction of \( \text{CH}_4 \) partial pressures within the flooded soil, thereby causing a reduction in dissolved \( \text{CH}_4 \) pore water concentrations and \( \text{CH}_4 \) 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 \( \text{CH}_4 \) oxidation in rice ecosystems will be affected by elevated soil temperatures under global warming. Three objectives were set: (1) determine stable hydrogen and isotopic compositions of \( \text{CH}_4 \) under different soil temperatures; (II) characterize seasonal patterns of \( \text{CH}_4 \) oxidation, and (III) use \( \delta^{13} \text{C} \) and \( \delta \text{D} \) to calculate fraction and \( Q_{10} \) of \( \text{CH}_4 \) 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 CH$_4$ 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 CH$_4$ 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, CH$_4$ was converted to either CO$_2$ (for δ$_{13}^C$) or H$_2$ (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 δ$_{13}^C$) or 2.3 (for δD). The isotope ratios were determined with reference to the standards V-PDB (for δ$_{13}^C$) and V-SMOW (for δD).

To calculate the fraction of CH$_4$ oxidized, the background isotopic composition was accounted for when calculating the isotope ratios (δ$_E$) for CH$_4$ emissions:

$$\delta_E = \frac{C_F \delta_F - C_I \delta_I}{C_F - C_I}$$

Where δ$_I$ and C$_I$ are the δ$_{13}^C$ or δD and concentration for the initial gas sample and δ$_F$ and C$_F$ refer to the δ$_{13}^C$ 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.

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 $\delta^{13}C$ of CH$_4$ in pore water from planted tubs (solid lines) and unplanted tubs (broken lines) for different temperature treatments.

Aboveground $\delta$D 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 $\delta$D, 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 CH₄ in planted tubs ranged from -64 ‰ to -47 ‰ δ¹³C 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 CH₄ 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 CH$_4$ 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 CH$_4$ 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 δ$^{13}$C-CH$_4$ and δD-CH$_4$ information (Fig. 6.6), the range of results also indicate that the main methanogenic pathway of CH$_4$ 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 $\delta^{13}$C and $\delta$D (Bergamaschi, 1997).

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.

The other methanogenic pathway for CH$_4$ formation (CO$_2$ reduction) is generally more depleted in $\delta^{13}$C (-110 to -60 ‰ V-PDB) and more enriched in $\delta$D (-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 $\delta^{13}C$ (~6 ‰) and $\delta D$ (~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 $\delta^{13}C$ were higher at higher temperatures (-59.0 ‰, -58.6 ‰, -58.0 ‰, -57.0 ‰, 20-32 °C) and $\delta D$ was lower at higher temperatures (-272 ‰, -296 ‰, -311‰, -331‰). This effect is not yet well understood.

6.4.2 Relationships between $\delta^{13}C$ and $\delta D$ in Emitted and Dissolved CH₄

Very high positive regression coefficients ($r^2 = 0.760$ to 0.957) were found between $\delta^{13}C$ and $\delta D$ 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 $\delta D$ value of CH₄ (which has been partially oxidized by bacteria) is 8-14 times greater than the change in the $\delta^{13}C$ value.
In general, all regression coefficients were positive, but were higher in lower temperature treatments. Similarly, positive correlations between δ\textsuperscript{13}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\textsubscript{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 $\delta^{13}C$ and $\delta D$ 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, $\delta^{13}C$ and $\delta D$ values in pore water from planted tubs.](image)

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 $\delta^{13}C$ and $\delta D$ 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 δ¹³C 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 δ¹³C values for planted tubs.](image)

Fig. 6.9: Fraction of CH₄ oxidized as determined from δD and δ¹³C values for planted tubs.
From δD values, the ranges in different temperature treatments were quite variable. The highest mean CH₄ 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 δ¹³C and δD-calculated oxidation fractions. To date, there is little information in literature about the dual use of these isotopes as tracers in CH₄ 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 αoxs for δ¹³C and δD. However, from δ¹³C-calculated oxidation fractions, the optimum soil temperature for CH₄ oxidation was found to be 28 °C, consistent with values of between 25 °C and 30 °C reported in Chanton and Liptay (2000). The Q₁₀ calculated from the fraction of CH₄ oxidized were 1-2 and 1.4-2.4 using δ¹³C and δD values, respectively.

6.5 Conclusion

This research clearly showed that temperature is an important factor in CH₄ oxidation in rice ecosystems. However, further empirical observations are needed to refine methods used in determining the transport fractionation. The calculated Q₁₀ values were less than average CH₄ fluxes and production. Such a scenario would promote increase in CH₄
production and emissions, resulting in a rising atmospheric CH₄ composition under global warming.
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 (\textit{mcrA}) and particulate methane monooxygenase (\textit{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 \textit{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 (\textit{mcrA}) and methanotrophs (\textit{pmoA}) were preferentially located to certain regions of the soil profile under different soil temperatures. \textit{mcrA} copies were positively correlated to temperature, whereas no significant response of \textit{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 CH$_4$ fluxes from rice agriculture. This result supports our work with isotopes described in the previous chapter.

7.1 Introduction

In rice fields, CH$_4$ is produced by three different pathways: carbon dioxide reduction, the methylotrophic pathway, and the acetate splitting pathway. The net CH$_4$ fluxes are controlled by the balance between microbial production and consumption of CH$_4$, and by physical processes such as diffusion, ebullition and ventilation. In anoxic freshwater environments, CH$_4$ 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 CH$_4$ 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 CH$_4$ 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 H$_2$ or CO$_2$ 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 methylotrophs 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.
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 UltraCleanTM 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 \textit{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 30 s, 56 °C for 30 s, 72 °C for 1 min, followed by a single cycle of 82 °C for 10 s. Plasmids containing the \textit{mcrA} gene were used as quantitation standards as previously described (Wilson et al., 2010). In a similar fashion, quantitation of \textit{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 \textit{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 (H₀) 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).
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 ng µL⁻¹ g⁻¹ (dry soil), with highest values in 5-15 cm soil depth (Fig. 7.5).
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 $5 \times 10^5$ to $3 \times 10^6$ 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.](image)

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 $5 \times 10^4$ to $4 \times 10^5$ 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 \textit{pmoA} copies at different soil horizons at different soil temperature treatment. Highest \textit{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 (O$_2$ supply into the rhizosphere) in different rice cultivars. A higher number of \textit{pmoA} copies was found in the aerobic top soil horizon (0-5 cm), an indication that methanotrophy is an aerobic process (Fig. 7.8).
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 \textit{pmoA} and \textit{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 \text{CH}_4 production and in the process, depletes \text{O}_2 and creates anaerobic conditions. The other reason for the low number of \textit{pmoA} copies is that the soils were amended with chemical fertilizers, which have been found to inhibit \text{CH}_4 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 \textit{mcrA} copies was located in the top soil layer (0-5 cm), while \textit{pmoA} copies was 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 \textit{pmoA} and \textit{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 CH$_4$ production zone (5-10 and 10-15 cm), well-defined stratification of \textit{mcrA} to \textit{pmoA} ratios was observed. This, according to our knowledge, is the first time these results have been obtained.

7.5.5 Regression between \textit{pmoA}, \textit{mcrA} Copies and Temperature

To gain insight into the dependence of \textit{pmoA} on \textit{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 \textit{pmoA} and \textit{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 CH$_4$ is produced, which in turn stimulates the growth of methanotrophs which depend on the CH$_4$ from methanogenesis as their substrates.
Fig. 7.10: Regressions: natural log (\textit{pmoA} copies g$^{-1}$ dry soil) versus natural log (\textit{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 0-20 cm soil depth.

Consistent with our results, Joulian et al. (1997) found methanotrophs to be positively correlated with methanogens within the main CH$_4$ 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 CH$_4$ 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).

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>0-5 cm depth</th>
<th>5-10 cm depth</th>
<th>10-15 cm depth</th>
<th>15-20 cm depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>$y = 0.1142x + 6.7884$</td>
<td>$y = 0.1082x + 6.2003$</td>
<td>$y = 0.657x + 0.5344$</td>
<td>$y = 0.6338x + 0.5795$</td>
</tr>
<tr>
<td>24</td>
<td>$y = 0.6984x + 1.462$</td>
<td>$y = 0.6298x + 1.0053$</td>
<td>$y = 0.9524x - 2.5185$</td>
<td>$y = 0.3679x + 2.6794$</td>
</tr>
<tr>
<td>28</td>
<td>$y = 0.0005x + 8.6413$</td>
<td>$y = 0.484x + 2.8714$</td>
<td>$y = 0.9142x - 2.096$</td>
<td>$y = 0.5019x + 1.6428$</td>
</tr>
<tr>
<td>32</td>
<td>$y = 0.1803x + 7.0367$</td>
<td>$y = 0.8161x - 0.257$</td>
<td>$y = 0.5079x + 2.8262$</td>
<td>$y = 0.3336x + 4.314$</td>
</tr>
</tbody>
</table>

7.5.6 Relationships between CH$_4$ Fluxes and *mcrA* and *pmoA* Copies

CH$_4$ fluxes increased with increasing abundance of *mcrA* copies (Fig. 7.11). The observed trends between CH$_4$ fluxes and *mcrA* copies were strikingly similar to those observed between CH$_4$ fluxes and *mcrA* gene transcript abundances reported in literature (Freitag et al., 2010; Freitag and Prosser, 2009).
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 CH$_4$ 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 CH$_4$ 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 CH$_4$ 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:

\[ Q_{10} = \left( \frac{P(T_0 + \Delta)}{P(T_0)} \right)^{10/\Delta} \]

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.](image)

The calculated $Q_{10}$ 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 $Q_{10}$ 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 $Q_{10}$ 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 CH$_4$ fluxes, and hence increase the global atmospheric CH$_4$ 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$_2$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 Reddy (1982).

In the soil, N$_2$O 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). Kham 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 CH$_4$ production
rates and pore water concentrations, respectively.

8.4 Results and Discussion

8.4.1 N$_2$O Fluxes

With the exception of the increase after N-fertilization, N$_2$O fluxes were generally low
for all temperature treatments. The high N$_2$O 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 N$_2$O 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 N$_2$O 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 N$_2$O emission into atmosphere is
suppressed by the pressure of standing water and therefore most of the N$_2$O produced
gets denitrified to N$_2$ within the soil, whilst under drier conditions, nitrification
produces most of the N$_2$O. If the soil is flooded again, denitrifying bacteria will act on
the nitrate released by nitrification to produce more N$_2$O (Ghosh et al., 2003).
Fig. 8.2: $\text{N}_2\text{O}$ 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.
High temporal and spatial variability in N\textsubscript{2}O fluxes was observed both within and between treatments. Two weeks after DAT, fluxes were low and highly variable, with negative N\textsubscript{2}O fluxes occurring on many occasions. The observation was reported in various studies (McHale et al., 1998; Bremer, 1996). N\textsubscript{2}O fluxes were two times higher in unplanted than in planted tubs.

A comparison of N\textsubscript{2}O emissions from planted and unplanted tubs indicated that higher fluxes were consistently found in unplanted than planted tubs. In particular, highest peaks of N\textsubscript{2}O 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 N\textsubscript{2}O 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 N\textsubscript{2}O emissions. To observe the real effect of temperature, only the time averaged N\textsubscript{2}O flux during the peaks were considered (Fig. 8.3).
Barnard et al. (2005) found that soil warming did not have large direct effects on $N_2O$ 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 (Koponena, 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 $N_2O$ the 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 $\text{N}_2\text{O}$ 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 $\text{N}_2\text{O}$. 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 $\text{N}_2\text{O}$ occurred at lowest temperature (20 °C), and lowest concentration at 32 °C. Increasing soil temperature reduces the pore water $\text{N}_2\text{O}$
concentration in the rhizosphere. Within all treatments, unplanted (control) tubs had the highest N$_2$O 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 N\textsubscript{2}O 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 N\textsubscript{2}O formation. Pore water concentrations were lower in higher soil temperature treatments than lower ones. For example, in both seasons, the N\textsubscript{2}O concentration at 20 °C was almost 15 times higher than that at 32 °C.

8.5 Conclusion

The direct effects of temperature on N\textsubscript{2}O emissions and production were successfully quantified. Spikes of N\textsubscript{2}O emissions coincided with period of nitrogen fertilizer application. Both N\textsubscript{2}O emission and production rates were dependent on soil temperature, with higher Q\textsubscript{10} values for fluxes than production. This may be the reason why very high N\textsubscript{2}O fluxes occurred just after flooding, and diminishing to zero elsewhere. The Q\textsubscript{10} 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 N\textsubscript{2}O production and emission rates in flooded rice paddies. Nevertheless, the quantity and distribution of future N\textsubscript{2}O emissions will depend on other indirect effects of global warming, such as the regional distribution of soil water content (water tables), and precipitation patterns.
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 Q₁₀'s 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.
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707.

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Appendix A

Seasonal average temperature data

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

<table>
<thead>
<tr>
<th>Date</th>
<th>WB1 (°C)</th>
<th>WB2 (°C)</th>
<th>WB3 (°C)</th>
<th>WB4 (°C)</th>
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### 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

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Appendix C

Average CH$_4$ and N$_2$O Production Data

Table C.1: Average CH$_4$ production data (mg/m$^2$/hr)

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Table C.2: Average N$_2$O production data (µg/m$^2$/hr)

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## Appendix D

### Pore Water CH$_4$ and N$_2$O concentrations

Table D.1: Average CH$_4$ pore water concentration (µg/L) data

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Appendix E

Average CH₄ Isotope Measurements

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

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

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Table E.4: Seasonal average δ¹³C values from pore water samples (per mil)

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