DIURNAL VARIATIONS IN METHANE EMISSION FROM RICE PLANTS

A Thesis

by

NICHOLAS AARON LASKOWSKI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Soil Science

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Approved as to style and content by:

James L. Heilman (Chair of Committee)	Kevin J. McInnes (Member)
Thomas W. Boutton (Member)	Mark A. Hussey (Head of Department)

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ABSTRACT

Diurnal Variations in Methane Emission from Rice Plants. (August 2004)

Nicholas Aaron Laskowski, B.S., Texas A&M University

Chair of Advisory Committee: Dr. James L. Heilman

A greenhouse study was conducted to investigate the mechanisms causing diurnal variations in methane emission from rice plants (*Oryza sativa* L.). Methane emission was measured using a closed chamber system on individual rice plants at five stages of development. The role of the rice plant as the primary methane transport component was examined by comparing emission from intact plants to plants severed above and below the water. No diurnal variations were present in the severed plants and the emission was greatly reduced when compared to the intact plant. Results from the vascular transport experiment showed that transpiration is a major factor in methane emission. Emission dependence on soil temperature was examined to test the hypothesis that soil temperature affects emission. With some plants, soil temperature was held constant using a water bath, otherwise the soil temperature was allowed to vary with environmental conditions in the greenhouse. Diurnal variations in emissions were higher for plants with uncontrolled soil temperature than for plants with controlled soil temperature. Soil temperature at a 5 cm depth explained 46% of the emission variation. Soil temperature affects the source of methane in the soil while transpiration promotes the uptake of water and subsequently the emission of methane. Methane emission was negatively correlated with biomass, probably due to effects of root biomass on soil water

methane concentration. Methane concentration in soil water was negatively correlated with root biomass, most likely due to increases in soil oxidation with increasing biomass in a fixed soil volume, and change in root conductance with age.

DEDICATION

To all those who have encouraged me in my academic journey, especially…

My wife Lindsay, who has been beside me and has encouraged me,

And my family for instilling good morals, a hard work ethic, and a never give up attitude.

ACKNOWLEDGEMENTS

For their guidance and support, I thank Dr. James Heilman, Dr. Kevin McInnes, and Dr. Boutton. Their encouragement enabled me to continue this project in the face of adversity. Dr. Heilman allowed me the freedom to try to implement new ideas and procedures and offered solutions when they failed to work. Dr. McInnes offered insight on construction and fabrication of sensors and instruments. Dr. Heilman and Dr. McInnes both had an open door policy that offered me a valuable reference of experience and information. To Dr. Boutton, I thank you for offering outside ideas and a perspective that opened my eyes to what I may have overlooked. It has been a pleasure to work with these fine individuals, for their character, intelligence, and personality made this two and half years a very memorable and productive period of my life.

To my fellow graduate students, Nathan Gardiner, Faith Ann Heinsch, Euclides Jasso, and all other students that have helped me along the way, I am grateful for your generous aid and advice throughout my research.

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INTRODUCTION

Methane is a potent greenhouse gas, 20 times more effective than carbon dioxide at absorbing longwave radiation (Watson et al., 1990). For this reason, methane concentrations and emissions have been heavily studied. Atmospheric concentrations of methane have been increasing at the rate of about 0.5% per year since the beginning of the industrial revolution (Bossio et al., 1999). Atmospheric methane concentrations have increased from 1.58 ppm in 1974 to approximately 1.72 ppm in 1999. About 10-30 % of global anthropogenic methane emissions (Denier van der Gon and Breeman, 1993; Houghton et al., 1990; and Neue and Sass, 1994) (Fig. 1) are from flooded rice paddies. Global methane emissions from rice paddies have been estimated to account for 60 to 170 Tg of carbon (Cicerone and Oremland, 1988). With an increasing population, particularly in Asia, more land is planted to rice each year to supply the demand. Since the majority of rice produced in the world is in flooded paddies, methane emission from rice paddies is expected to increase. Researchers are currently trying new management strategies that might mitigate some of the methane emission from rice fields.

Methane emissions from rice paddies are largely influenced by rates of microbial production and oxidation of methane (Conrad, 1993), plant growth stage, and environmental conditions (Watanabe et al., 2001). There are three pathways available for escape to the atmosphere. These pathways are: 1) molecular diffusion, 2)

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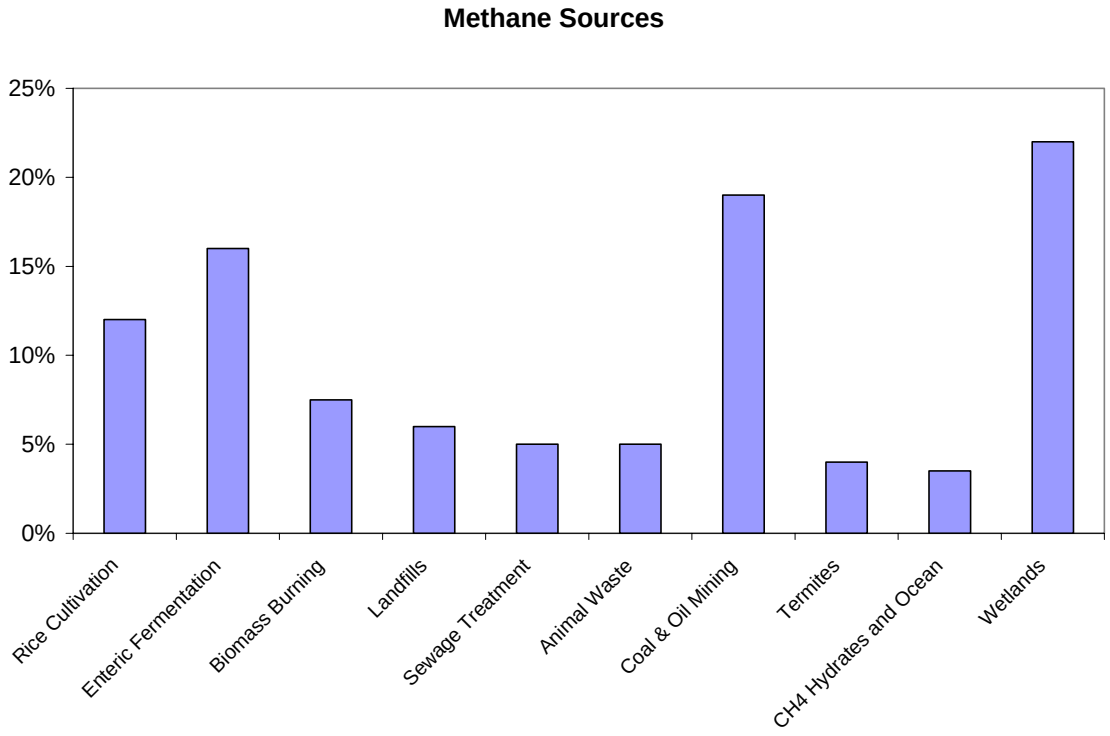

Figure 1. Global methane emission (adapted from USDA-FAS, 2002)

ebullition of methane, and 3) methane transport through the rice plant (Cicerone and Shetter, 1981; Inubushi et al., 1989; Neue et al., 1994 and Schutz et al., 1991). Of these pathways, it has been estimated that more than 90% is released through the rice plants (Neue et al., 1994; Schutz et al., 1991). The ability for rice to grow in anoxic soil is because it possesses a well developed system of air spaces (aerenchyma) that supply atmospheric oxygen to the roots for respiration. These aerenchyma allow methane transport from the roots to the atmosphere.

There have been many studies of methane transport through the rice plant, but it has only been in the last few years that the importance of methane as a greenhouse gas has been understood. Studies have addressed the production and oxidation of methane in the soil (Takai, 1970; Kruger et al., 2001; Eller and Frenzel, 2001). Researchers have also investigated the effects of growth stage (Watanabe et al., 2001; Yang and Chang, 1999; Yao et al., 2000), plant variety (Yao et al., 2000; Mitra et al., 1999), soil temperature (Wang et al., 1997; Hosono and Nouchi, 1997; Watanabe et al., 2001), and physiology (Nouchi et al., 1990; Yao et al., 2000) on methane emission.

Understanding the mechanisms that control methane emission allows prediction of emission rates. Diurnal and seasonal variations in methane flux from rice paddies have been found in many studies. However factors that cause the variation are unclear. The most prevalent hypothesis is that diurnal variations in soil temperature are the cause of diurnal variations in emission. Positive correlations of methane fluxes and soil temperatures have been reported by Schutz et al. (1989), Wang et al. (1997), Yagi and Minami (1993), Neue and Sass (1994), and Sass et al. (1994), while others have found

no correlation (Cicerone et al., 1983; Yagi and Minami, 1993; Wang et al., 1993; Chen et al., 1993). Hosono and Nouchi (1997) found that methane emission was correlated with the soil temperature at a depth of 5 cm beneath the water soil interface. Watanabe et al. (2001) found that maximum emission occurred during the middle to late stages of rice growth and attributed emission primarily due to temperature and not growth stage development.

Research objectives

The phenomenon of diurnal variation of methane emission in rice has been investigated many times. I will test the hypothesis that soil temperature variation contributes to diurnal methane emission. The objectives of my research are to 1) to determine if a diurnal pattern in methane emission exists, and 2) to determine if the cause of this diurnal variation is soil temperature.

LITERATURE REVIEW

Atmospheric methane concentrations

Global methane emissions from rice paddies have been estimated to account for 60 to 170 Tg of carbon annually (Cicerone and Oremland, 1988). With an increasing population, particularly in Asia, more land is planted to rice each year to supply the demand. Since the majority of rice produced in the world is in flooded paddies, methane emission from rice paddies is expected to increase. Researchers are currently trying new management strategies that might mitigate some of the methane emission from rice fields. Such as temporary paddy drainage during the latter periods of vegetative propagation.

Methane production

Methane is generated biologically by methanogenic bacteria, a major division of the Archaea kingdom. Environments suitable for methanogenesis are very reduced (<-150mV, Oxidation Reduction Potential) and are typically found in flooded ecosystems. Substrates for methanogenesis come largely from acetate dissimilation and to a lesser extent carbon dioxide reduction (Cicerone and Shetter, 1981 and Krugeret al., 2001). Dunfield et al. (1993), Koyama (1963), and Lindau et al. (1993) have documented that

methanogenesis in the soil is dependent on soil temperature, microbial substrates, and anoxic conditions. Methane production has a Q_{10} of 4.6 which is much higher than pther microbial processes in reduced sediments. Methane production requires an oxygen-free

environment and is affected by availability of substrates. Both quality and quantity of organic matter affects the ease of utilization by methanogens (Chen et al., 1993).

Methane transport pathways

Methane produced in a flooded rice paddy has three pathways available for escape to the atmosphere (Fig. 2). These pathways are: 1) molecular diffusion, 2) ebullition of methane, and 3) methane transport through the rice plant (Cicerone and Shetter, 1981; Inubushi et al., 1989; Neue et al, 1994; and Schutz et al, 1991). About 90% of all methane emitted from the rice paddy is through the plant from anoxic paddy soil (Cicerone and Shetter, 1981; Inubushi et al., 1989; Neue et al, 1994; and Schutz et al, 1991). Mode of oxygen transport is through the aerenchyma which also serves as a conduit for methane transport from the soil water to the atmosphere. Hosono and Nouchi (1997) proposed that both the oxygen and methane movements are mediated by molecular diffusion down a concentration gradient.

Rice plant emission rates

Methane emission from rice typically has been measured using the closed chamber method in which the flux is determined from the rate of change in methane concentration in the chamber. Yagi and Minami (1993) measured emission rates in a rice plot in Japan and found it ranged between 2.9 and 70.1 mg of C m^{-2} h^{-1}. Adhya et al. (1994) found emissions ranged from 4 to 26 mg of C m^{-2} h^{-1} in a rice paddy, and Mitra et al. (1999) found hourly emissions ranged from 0.65 to 1.12 mg of C m^{-2} h^{-1}. Average emission values are approximately 21.4 g of C m^{-2}, depending upon plant variety and growth environment.

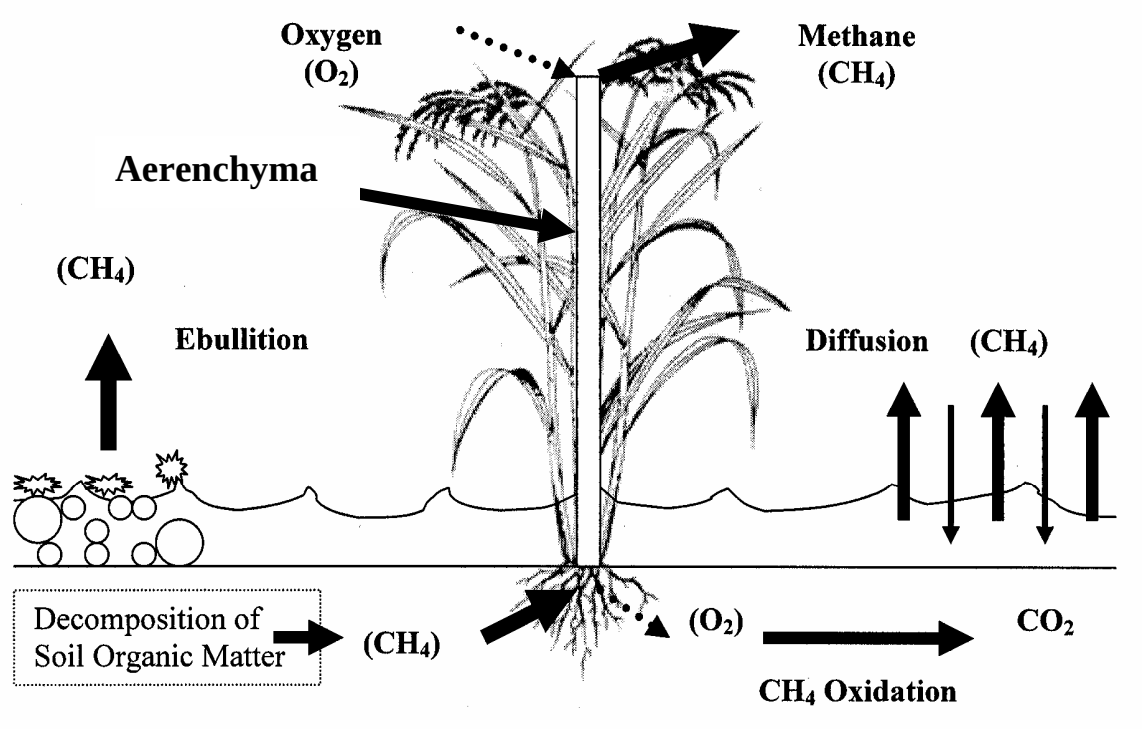

Figure 2. Pathways of methane movement from soil to the atmosphere

Environmental and plant variables significance to emission

Many environment and plant variables affect methane emission. Environmental variables include temperature, rainfall, soil texture, organic matter, and red-ox potential of the soil. Plant variables are related to the ability to conduct methane from the root soil interface to the atmosphere. Yao et al. (2000) and Sass et al. (1990) found that plant methane conductance through the aerenchyma increases with size and age of the plant. Larger plants have the ability to conduct more oxygen and methane through their aerenchyma because larger, more mature plants have more developed aerenchyma. Positive correlation of emission with biomass, number of tillers, and shoot height has been shown by Ding et al. (1999) and Huang et al. (1997). However, Watanabe et al. (1995) showed no correlation between emission and any plant biomass parameters. Hosono and Nouchi (1997) found that root conductance of methane varied with temperature, and was twice as high at 30º C than at 15º C and root conductance was shown to decrease with age and size (Singh et al., 1999).

Effect of temperature on emission

Wantanabe et al. (2001) attributed the variability of methane emission in rice paddies to air temperature. He hypothesized that elevated plant temperatures cause transpiration to increase, resulting in more water exchange with the atmosphere causing more soil water coming in contact with the rice plants roots. He also found very little correlation with soil temperature and emission. Cicerone et al. (1983), Yagi and Minami (1993), Wang et al. (1993), and Chen et al. (1993) also found little correlation between soil temperature and emission and attributed the variation in emission to fertilization

practices and increased root exudates which serve as substrates for methanogenesis. Sass et al. (1994) found seasonal variation in methane production and emission followed plant development with no apparent seasonal temperature dependence.

Soil temperature variation

Root zone temperature variations have been shown by Schutz et al. (1989), and Yang and Chang (1999), and are correlated with air temperature. However, heat flow to soil depths has not been examined in a rice paddy. Natural temperature regimes in rice paddies located in the southern United States range from 21 to 26 ^{o}C at a 5 cm soil depth (Schutz et al. 1989) while Yao et al. (2000) reported a much lower temperature variation of 25.2 to 28.1 ^{o}C.

Diurnal emission variation

Diurnal variations in methane emission have been found in rice paddies by Yang and Chang (1999), Schutz et al. (1989), and Buendia et al. (1997), with emission at a maximum during late afternoon and at a minimum in early morning. Wang et al. (1997), Schutz et al. (1989), Yagi and Minami (1993), and Neue and Sass (1994) showed rice plant emission of methane was positively correlated with soil temperatures at depths between 0 and 15cm. Schutz et al. (1989) showed differences in diurnal emission at three different growth stages, at naturally occurring soil temperatures, indicating that soil temperature accounts for more variability than growth stage.

The literature contains contradictory information on the mechanisms of diurnal variation in methane emission form rice. I intend to test the hypothesis that soil temperature variation is the mechanism affecting the diurnal variation in methane

emission by comparing methane emission under normal temperature variation with that occurring with static soil temperatures.

MATERIALS AND METHODS

The experiment was conducted in the greenhouses on the second floor of the Norman Borlaug Center for Southern Crop Improvement, Texas A&M University. Experiments began in January 2004.

Soil collection and fertilization

Soil from the Eagle Lake area (Texas), a Crowley Series fine sandy loam (Fine montmorillinitic, thermic Typic Albaqualf), was collected in bulk from the top 20 cm of top soil. The soil was mixed in a potting soil mixer with an addition of 12,000 kg ha^{-1} chopped rice straw and placed in 19L buckets. Fertilizer was applied at three different times with a total of 190 kg ha^{-1} nitrogen, a combination of NH_4NO_3 and urea, 40 kg ha^{-1} P_2O_5, and 50 kg ha^{-1} K_2O. Twenty five percent of the nitrogen was added pre-plant along with all the P_2O_5 and K_2O at a depth of 5 cm. Fifty percent of the nitrogen was added just prior to flooding on the dry soil and the remaining twenty five percent was applied at the panicle differentiation stage.

Plant germination and growth

Seed from the rice variety Cocodrie was germinated in fritted clay and then transplanted individually into the buckets at the 4^{th} leaf stage. The soil was flooded to a depth of 5 cm with distilled de-ionized water after transplanting and remained flooded throughout the growth season. Algae growth was minimized by using aluminum foil to cover the exposed water around the plant. Plants were allowed to grow naturally in a temperature controlled greenhouse.

Plant contribution to methane transport

Evaluation of the role of the rice plant in methane transport was examined. Methane emissions and soil water methane concentration of an intact rice plant was compared to plants severed both above and below the water level. Above ground biomass of the plant was removed approximately 4 hours prior to first sampling period. Plants were cleanly cut and not crushed, allowing for aerenchyma to function normally. A replication of this experiment was done.

Soil temperature control on methane emission

Methane emissions and soil water methane concentrations of plants at different soil temperature regimes were compared during three growth stages. During each growth stage the soil temperature for two plants were allowed to vary with the ambient environmental conditions in the greenhouse. In other plants, soil temperature was controlled by a water bath set at 24 ^{o}C (Fig. 3). The plants and soil were given 24 h for temperature equilibration**.**

Plant biomass and corresponding emissions

Plant biomass was compared to corresponding emission during their relative growth stages. Above and below-ground biomass was examined for its contribution to emission and soil water methane concentration. Seasonal methane emission and soil water concentration was plotted and discussed.

Sampling procedure

Methane emission measurements were made at five different growth stages (initial tillering, maximum tillering, maximum leaf area, anthesis, and maturity). During

Figure 3. Photograph of soil water bath

each growth stage emission measurements were taken every four hours (400, 800, 1200, 1600, 2000 and 2400 hours) totaling 6 periods per day using the closed chamber method. Gas emission concentrations and soil water samples were taken each period for three plants in the same growth stage. Replications were made during the sampling of the vascular contribution of the rice plant however, replications of temperature controlled experiments were not accomplished.

Closed chamber

The chamber (0.92 m height, 0.29 m diameter) was constructed of a clear poly-acrylic plastic so that it fit snugly over the rims of the buckets (Figs. 4 and 5). A small brushless fan was installed in the top of the chamber to mix the air.

Gas emission sampling

Gas samples from the closed chamber were collected manually every five minutes for a total of twenty minutes, creating a concentration increase with time. Methane flux density was calculated by the equation,

$$\text{Flux (g of C m}^{-2}\text{ s}^{-1}) = \Delta C/\Delta t * [\rho_{CH_4} * (V_{chamber}/ A_{surface\ area}) \quad [1]$$

where $\Delta C / \Delta t$ is the time rate of change in methane concentration, ρ_{CH_4} is density of methane corrected for temperature, $V_{chamber}$ is volume of chamber plus bucket headspace from the water surface to the top of the bucket, and $A_{surface\ area}$ is area of the water surface in the bucket.

Air samples of 500 μL were removed from the chamber every 5 minutes for a total of 20 minutes and manually injected into a gas chromatograph (SRI model 9610,

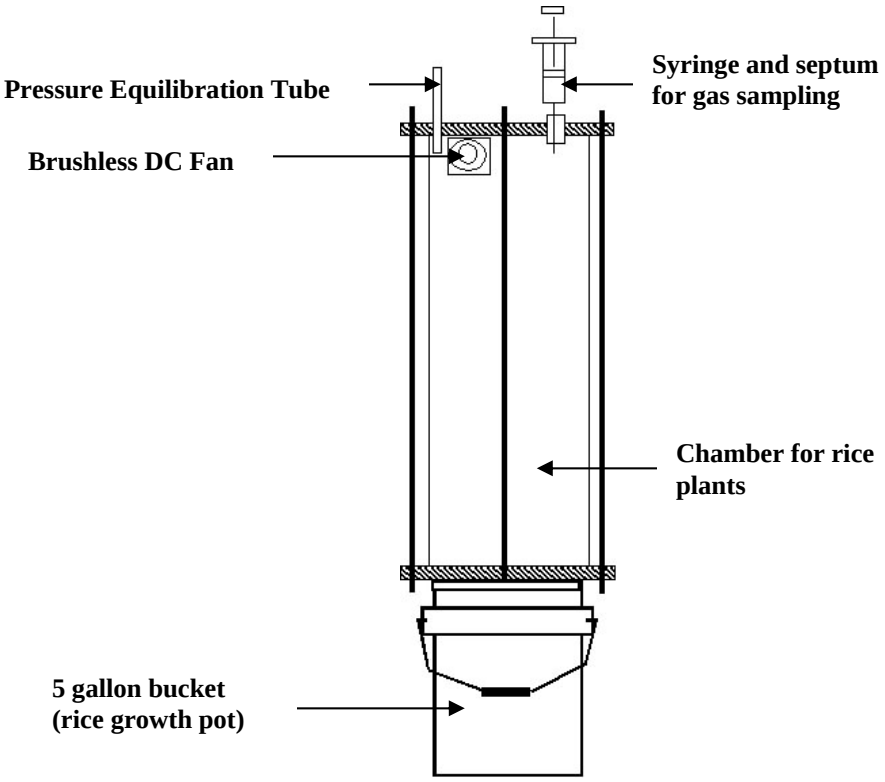

Figure 4. Design of the methane sampling chamber

Figure 5. Photograph of methane chamber on a rice plant

Torrance, CA) equipped with a flame ionization detector (FID) and a 6 foot stainless steel Haysep type D column, 80/100 mesh (Fig. 6). The column was held at 70 ^{o}C. Flow rates of air, helium (carrier gas) and FID hydrogen were 400 mL min^{-1}, 30 mL min^{-1} and 40 mL min^{-1}, respectively. Peak integration was accomplished automatically by the SRI's Peak 2 integration program. Retention time for methane was approximately 0.38 minutes.

Soil water sampling

Soil water samples were taken each period to determine methane concentration in the soil water. Soil solution samplers (Rhizon, Netherlands) were inserted through the sides of the buckets at the surface water, and a depth of 5, 10 and 15 cm (Fig.7). Soil water samples were removed using 10 mL draw blood collection tubes, (BD Vacutainers, Franklin Lakes, NJ). Processing of the soil water samples occurred within 24 hours of sampling and all samples were kept in a refrigerator at 3 ^{o}C prior to processing. The vacutainers were vigorously shaken for 30 s on a vortex machine, allowed 5 min for gas water equilibration, then 100 μL air samples were removed using a 1 mL gas tight syringe and manually injected into the GC (Alberto et al., 2000). Methane soil water concentration was determined using the equation,

$$X_L(\mu g/mL) = (X_a[V_a+\alpha V_L] - X_a{}^{o}V_a)/V_L \qquad [2]$$

where X_L is the concentration of methane (μg mL^{-1}), X_a is the concentration of methane (μg mL^{-1})in the headspace after shaking, $X_a°$ is the concentration (μg mL^{-1})prior to shaking (i.e. concentration of the ambient air), V_a is the volume (mL) of the headspace given by $(V_t - V_L)$, V_L is the volume (mL) of solution given by $(M_s - M_e)/\rho_L$, V_T is the

Figure 6. Photograph of GC lab equipment

Figure 7. Photograph of soil water sampling

volume (mL) of the vacutainer with cover given by $(M_f - M_e)$, M_e is the mass (g) of the empty vacutainer with cover, M_f is the weight (g) of the vacutainer filled with water, M_s is the weight (g) of the vacutainer and solution, α is the methane: water partition coefficient at $25\ ^\circ C$ is = 0.03, and ρ_L is the density of the solution at $25\ ^\circ C$ = 1.0 (g mL^{-1}). M_s is the weight (g) of the vacutainer and solution, α is the methane: water partition coefficient at $25\ ^\circ C$ is = 0.03, and ρ_L is the density of the solution at $25\ ^\circ C$ = 1.0 (g mL^{-1}).

Measurement and precision

All injections were done manually using 1 mL TB syringes. A 72.1 L chamber was flushed with 300 μL mL^{-1} methane, a sample set of 10 injections were with drawn and resulted in a mean of 302.5 μL mL^{-1} with a standard deviation of 2.7 μL mL^{-1}. A water sample was bubbled with 1102 μL mL^{-1} methane for five minutes and sealed, a sample set of 10 samples yielded a mean of 1107.52 μL mL^{-1} with a standard deviation of 66.5 μL mL^{-1}. Average change in methane concentration in the chamber was 18.76 μL mL^{-1} per period and average soil water methane concentration was 12144.4 μL mL^{-1}. The level of accuracy and precision allowed verification and significance of the all methane sampling data.

Environmental monitoring

Air temperature, water temperature, and soil temperature at depths of 5 cm, 10 cm, and 15 cm were measured with thermocouples inserted into their respective depths. Plant canopy temperature was measured using an infrared transducer and air temperature

was measured with a fine wire thermocouple. Temperature data were recorded using a data logger (Campbell Scientific CR23X, Logan, UT).

Plant biomass

Plants were destructively sampled the day after methane emission measurements to determine biomass. Collection of data included root weight, tiller number, total plant weight, height, and leaf area for each plant according to the procedure outlined by Nouchi et al. (1990).

RESULTS AND DISCUSSION

Methane transport in the rice plant

Plant mediated transport of methane accounts for approximately 90% of all emission pathways (Cicerone and Shetter, 1981; Inubushi et al., 1989; Neue and Sass, 1994; and Schutz et al, 1991). Contributions of the vascular portions have indicated that the aboveground biomass has an affect on emission. Dissections of the intact plant revealed that emissions were greater than those of plants severed below or above the water (Fig. 8). The intact plant displayed normal diurnal methane emission variation and severing the plant considerably reduced diurnal variation, error bars indicate the standard deviation in emission. Methane gas diffuses from the roots through aerenchyma to the leaf sheath and out into the atmosphere. It might be expected that the plant severed above the water would have a higher emission than the intact plant because the removal of tissue would decrease flow path resistance. The fact that emission was lower in the severed plants than the intact plant suggests that transpiration may play a major role in methane emission. Removal of the aboveground biomass that functions as part of the transpiration pathway greatly reduces transpiration and subsequently water movement from the soil to the atmosphere. Methane emission was lowest in the plant severed below the water most likely because the direct connection between the soil and the atmosphere was broken. In that case diffusion and ebullition were the only transport mechanisms.

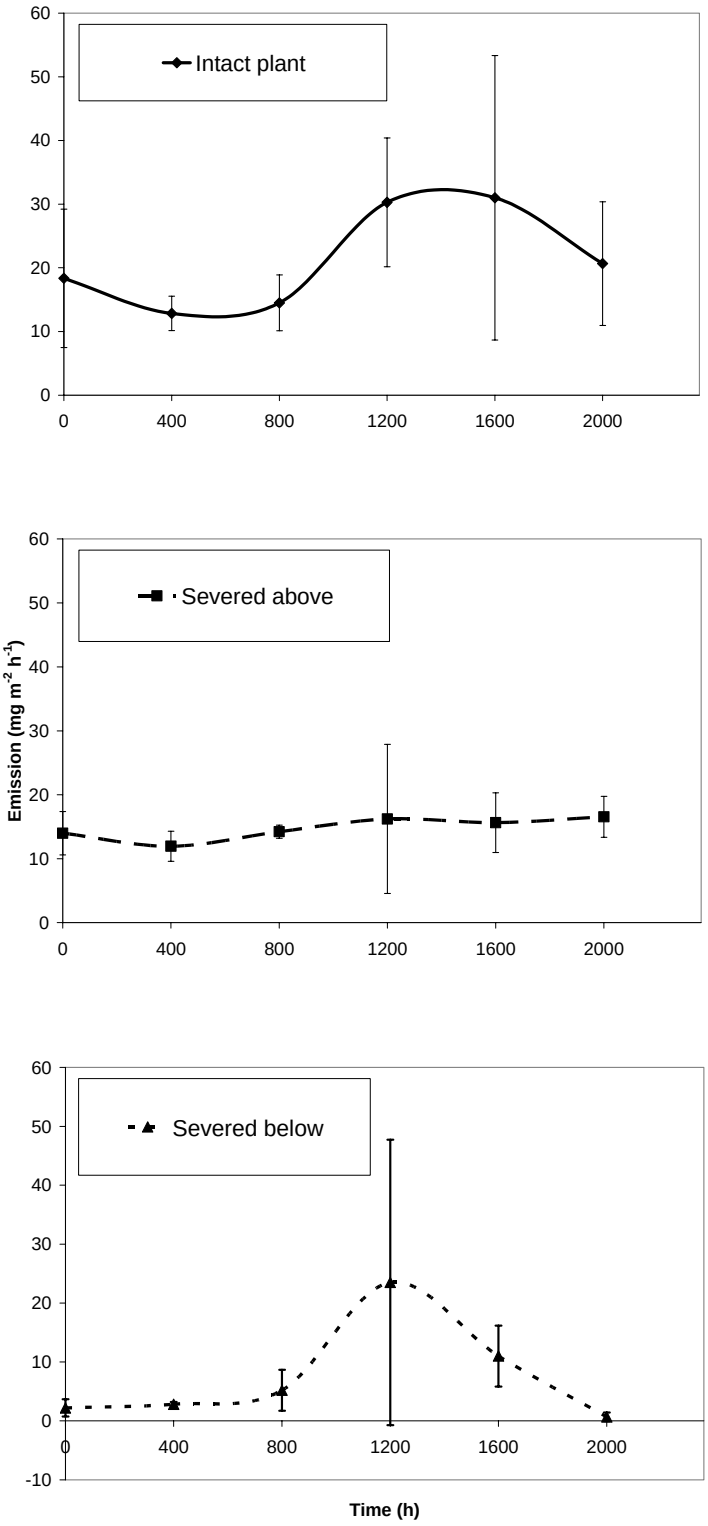

Figure 8. Effect of plant transport on methane emission

The effect that plant tissue has on methane transport is apparent. Seiler et al. (1984) and Nouchi et al. (1990) found that stomata and the transpiration pathway do not transport significant amounts of methane. Methane has not been found to transport through the plants xylem as a result of water movement and methane is not emitted through the stomates. Although not measured, transpiration indirectly effects methane emission. Simply, methane is contained in the soil water which is taken up by the plant as a function of water potential, once this methane is in the plant the methane is degassed, travels through the aerenchyma and exits from the plant near the leaf sheath into the atmosphere

Methane dissolved in the soil water is the source of the methane that escapes to the atmosphere. Methane concentration in the soil is dependent upon many variables that will be discussed in the next section however, soil temperature has been shown to have the greatest affect on methane production (Bodegom and Stams, 1999). Variations in soil temperature for all buckets were similar and followed a diurnal pattern. Diurnal variations in soil water methane concentration were found (Fig. 9).

The rice plant plays a major role as a conduit for methane transport. I found that direct effects of plant tissue may have a large affect on methane emission. Soil temperature was similar for all plants during the respective period, therefore methane production could have been controlled by another factor other than temperature. Possibly the rate of emission affected the methane source strength. Both variables are important factors that contribute to methane emission rates. It was found that plants do significantly affect methane emission rates.

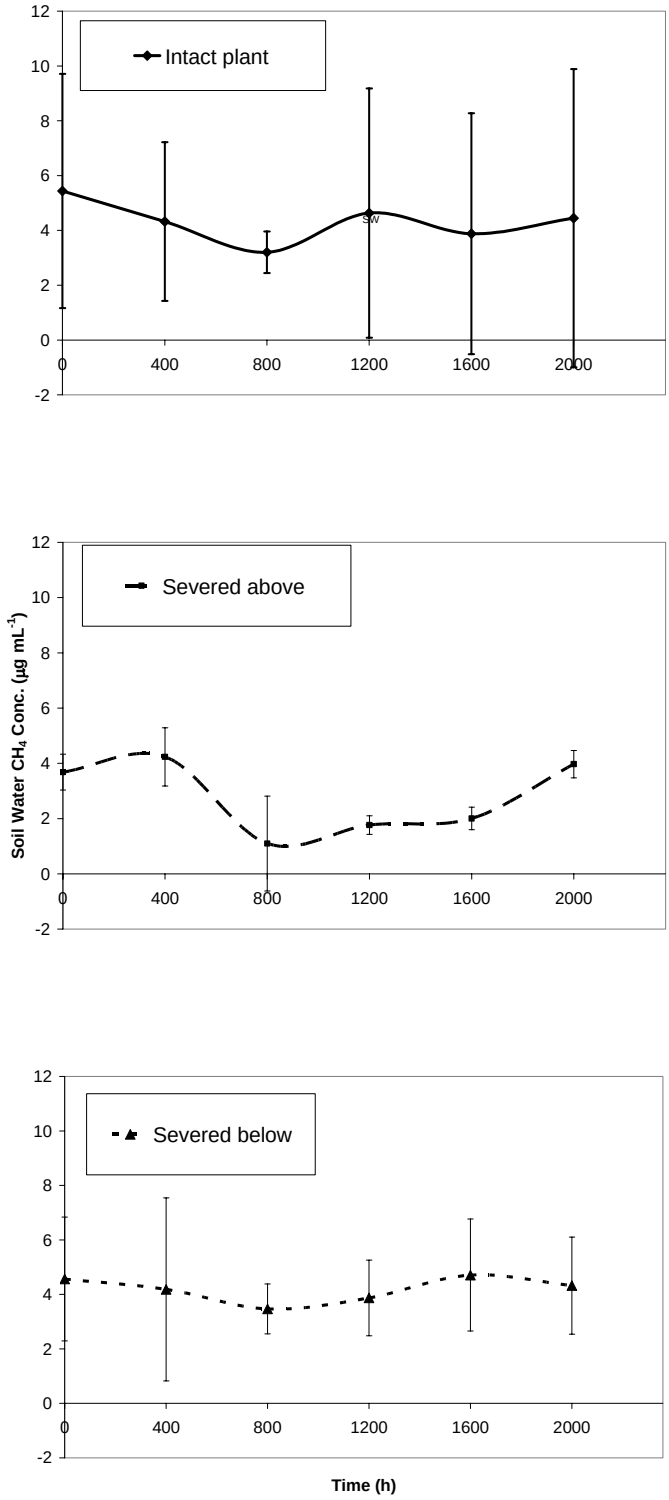

Figure 9. Effect of plant transport on soil water methane concentration at 5 cm

Diurnal methane emission and soil temperature

Diurnal temperature variation at a 5 cm depth has been noted as the depth that is most significant to methane emission (Wang et al., 1997, Schutz et al., 1989 and Yagi and Minami, 1993). Diurnal temperature variations at 5 cm were present at initial tillering, maximum tillering, and anthesis for plants with uncontrolled and controlled soil temperatures (Fig. 10). Except at initial tillering, soil temperature variations for controlled plants were held to ~1 ^{o}C. Diurnal soil temperatures at the 5 cm depth for uncontrolled treatment ranged from 23 to 29.9 ^{o}C (Fig. 11). This amount of variation present in the greenhouse (Table 1) was slightly greater than what has been recorded in rice paddies by Schutz et al. (1990) and Yao et al. (2000). The difference can be attributed to thermal warming of the side of the bucket from solar radiation. The larger variation was not a problem because it amplified the difference in diurnal variation between the temperature controlled plant and the non-temperature controlled plants.

Diurnal variations in methane emission occurred at all growth stages that were tested (Fig. 12). Amounts of emission variation varied from period to period with a minimum emission variation just prior to sunrise and maximum emission variation at noon (Fig. 13). Maximum emission occurred during the middle of the day and minimum emission occurred just prior to sunrise, as found by Yang and Chang (1999), Schutz et al. (1989), and Buendia et al. (1997). Plants with the controlled soil temperature had less diurnal variation in emission than those plants with no temperature control. Plant canopy and air temperatures were similar for controlled and uncontrolled treatments, indicating that soil temperature was a significant factor causing the diurnal variation.

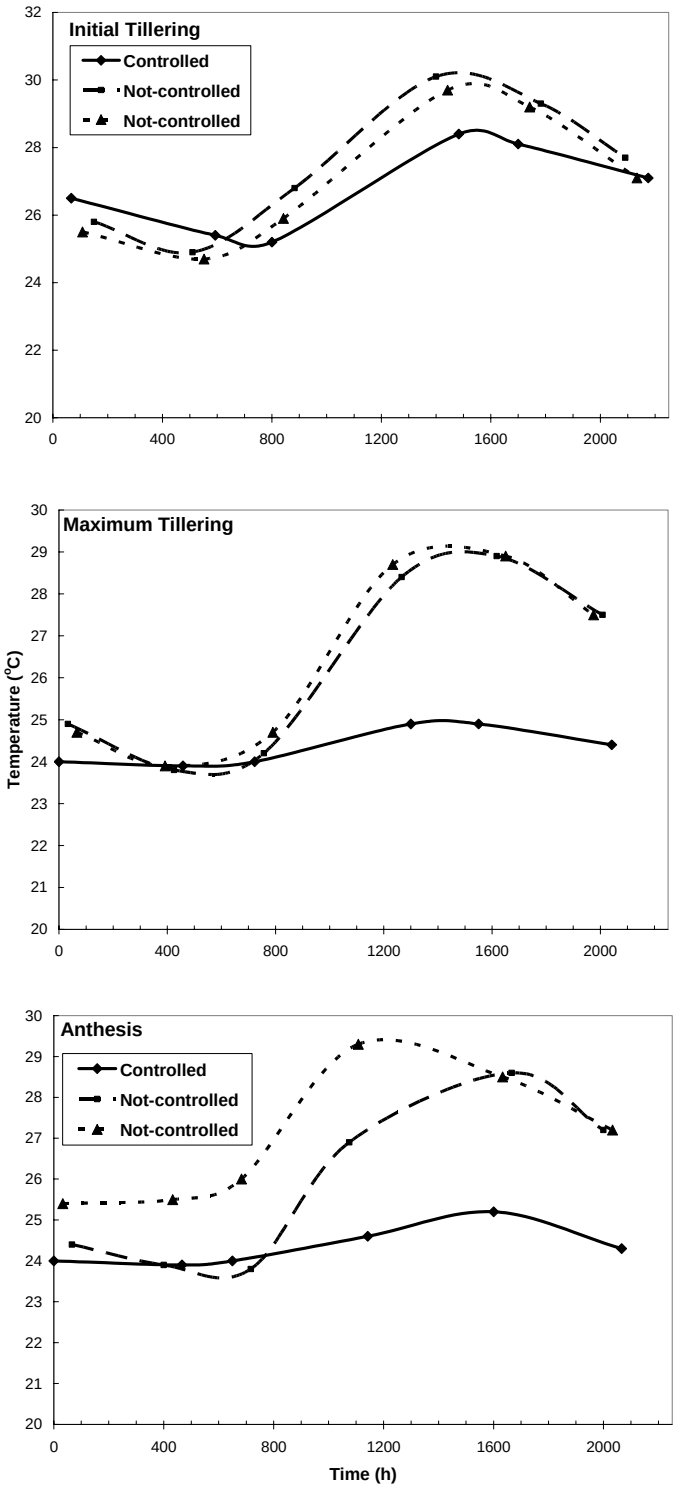

Figure 10. Representative diurnal temperature variation at 5 cm depth

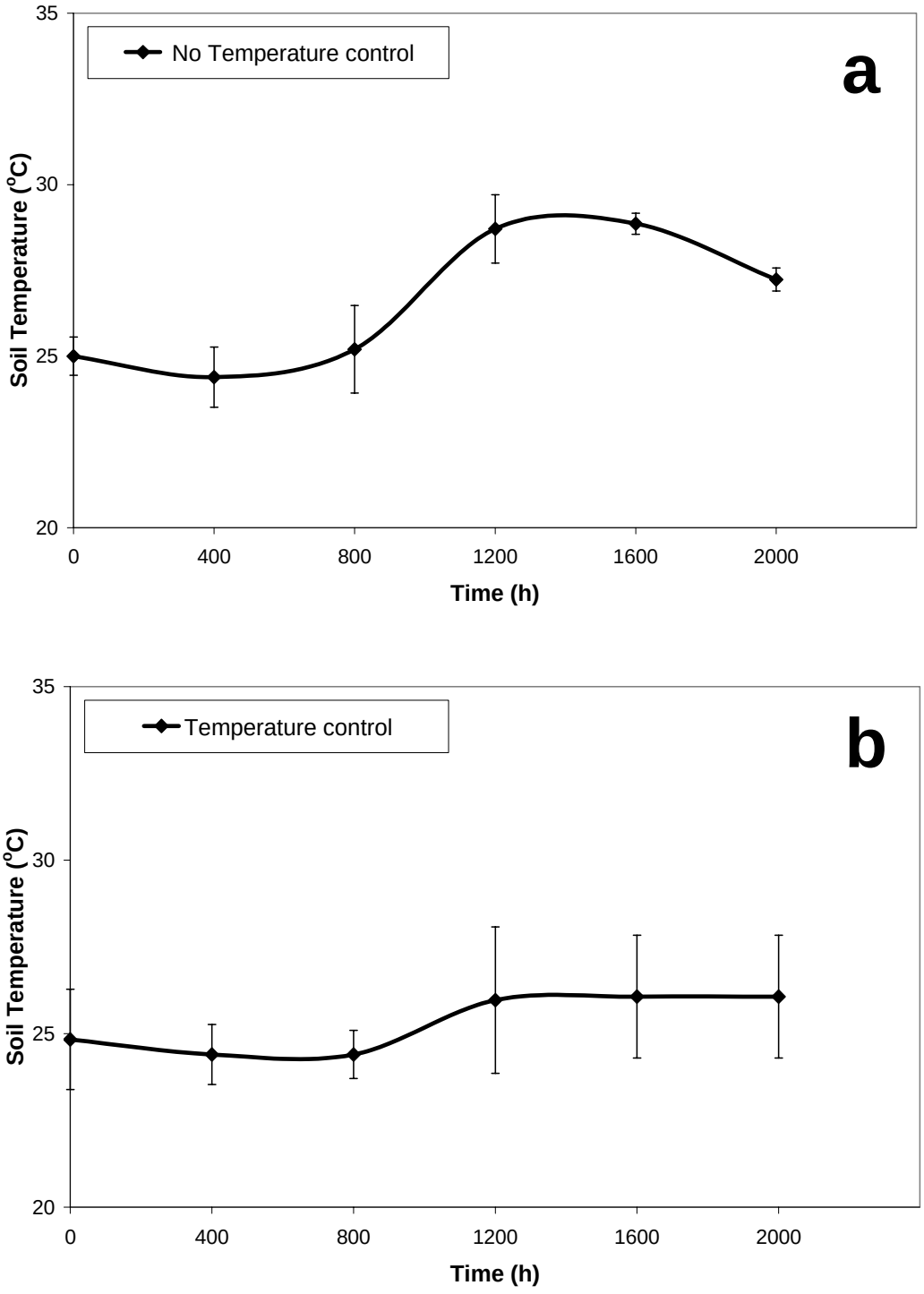

Figure 11. Soil temperature variation at 5 cm depth, no temperature control (a) and temperature control (b)

Table 1. Maximum and minimum air, soil, and plant temperatures for plants with uncontrolled soil temperature

	Air Temperature		Soil Temperature at 5 cm		Canopy Temperature	
Growth Stage	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
			°C			
Initial Tillering	32.6	24.5	29.9	24.8	34.3	35.9
Maximum Tillering	33.1	24.3	28.9	23.9	39.4	25.7
Maximum Leaf Area	32.5	24.7	29.0	25.1	34.4	26.0
Anthesis	35.8	24.4	29.0	24.6	32.3	26.2
Maturity	34.2	24.2	28.5	23.0	39.3	25.7

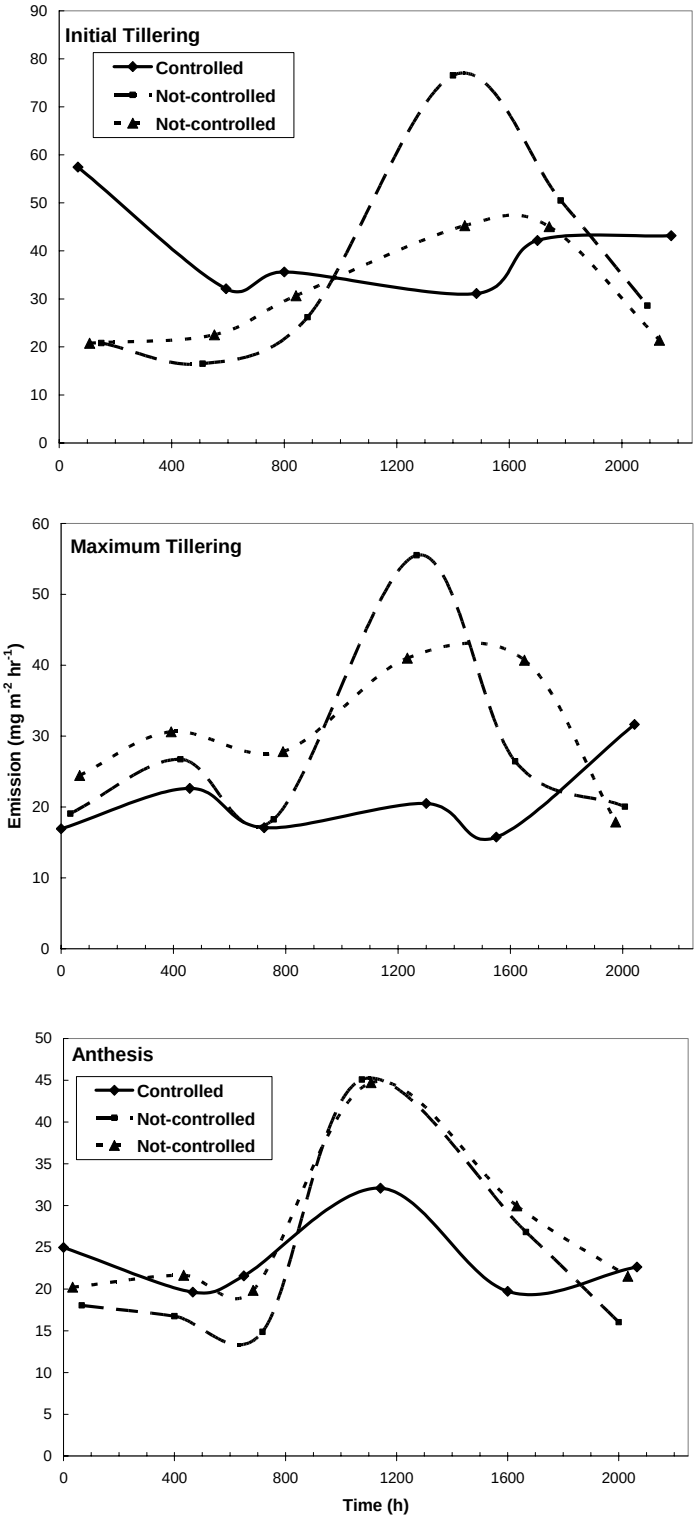

Figure 12. Representative diurnal emission variation

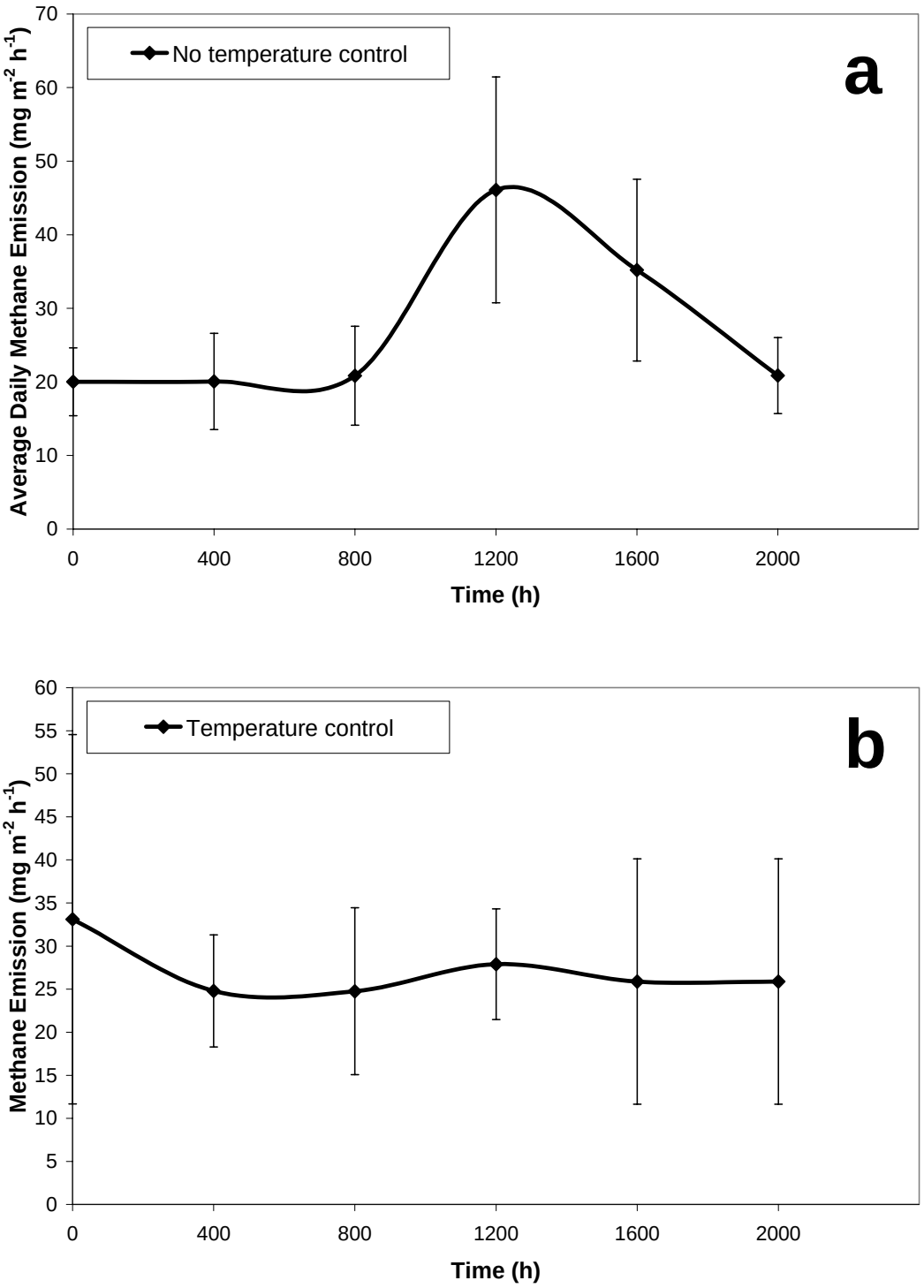

Figure 13. Diurnal methane emission variations, no temperature control (a) and temperature control (b)

Regression analysis showed that 48% of the variation in methane emission was explained by soil temperature at 5 cm (Fig. 14). Correlation strength between emission emission and soil temperature decreased with depth.

Soil temperature can affect emission by affecting conductance at the root, methanogenesis, and the amount of methane gas that can be dissolved in water.

Hosono and Nouchi (1997) found that root conductance of methane increased with temperature, due to the decrease in resistance to flow in the root epidermal cells. Methane production increases with increasing soil temperature because kinetic inhibition of metabolic activity is overcome by increasing energy in the system. As mentioned previously the Q_{10} for methanogenesis is 4.6 the data in Figure (Bodegom and Stams, 1999). Increasing water temperature also decreases the amount of methane gas that can remain in solution. As the soil temperature increases the methane held in the water degasses and is released as bubbles that come in contact with roots where the gas is absorbed, or are lost via ebullition (Albert et al., 2000).

The comparison of methane emission from plants with uncontrolled and controlled temperatures suggests that soil temperature contributes to diurnal variation in methane emission. This was indicated because plants with controlled soil temperatures have less diurnal variation in emission than plants with uncontrolled soil temperatures. The effect of increased soil temperature is thought to increase methane concentration in soil water and the increase of conductance at the rice roots. However, the temperature effect on emission is contradicted by the comparison of emission between the intact plant and that of the plants severed above the water line (Fig. 8) where plant tissue

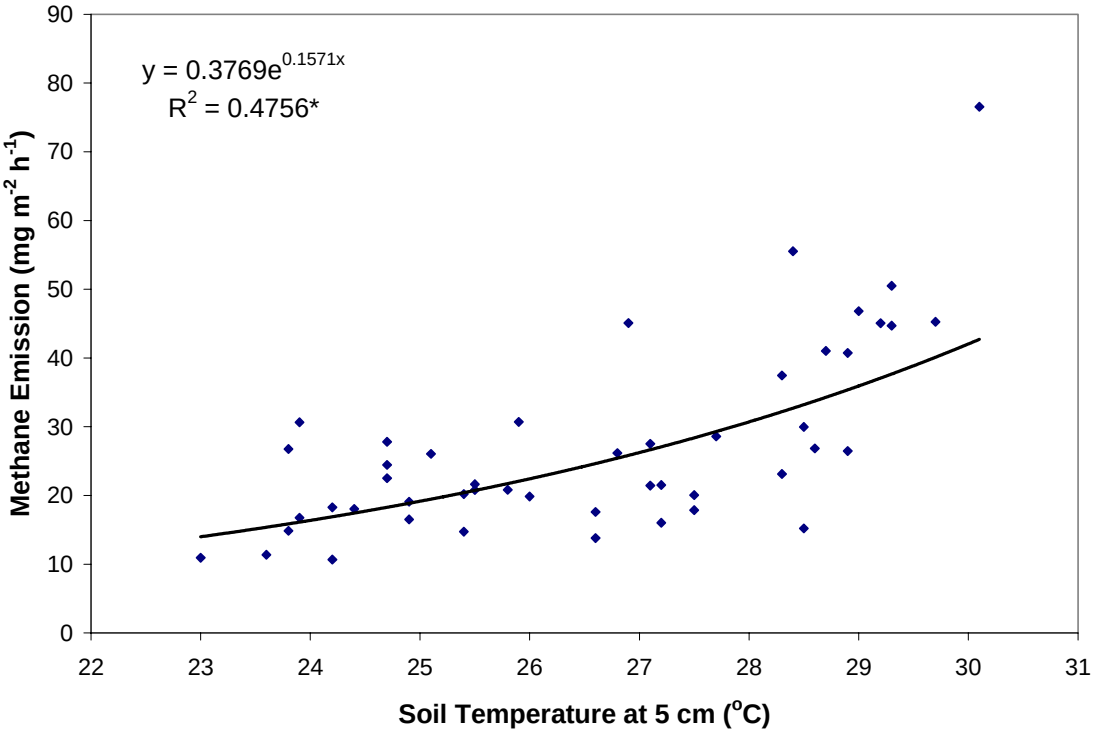

Figure 14. Emission vs soil temperature at 5cm depth.
*** denotes significance at the 0.10 level**

contributes indirectly by the plant process of transpiration and was suggested to be a major factor contributing to diurnal variations in emission.

Soil temperature has been found to be a major factor controlling soil water methane concentration (Bodegom and Stams, 1999) however, this is contradictory to what the data had shown (Fig. 15). It is unclear what caused the conflicting results of in production and soil water methane concentration. The factors controlling methane production and subsequently soil water methane concentration at 5 cm are not clearly defined. There appears to be no direct correlation with soil temperature at 5 cm and soil water methane concentration at 5 cm. Evidence of the temperature effect on root conductance can be inferred from the positive correlation between emission and soil temperature at 5 cm (Fig. 14). Increasing root temperature decreases root resistance to methane transport (Hosono and Nouchi, 1997). Some evidence has shown that plant temperature does explain some of the emission variability (Fig. 16) possibly due to the indirect effects of transpiration however, this can not be verified because transpiration was not measured.

Methane emission and biomass

Previous researchers found that methane emission increased as biomass increased and growth stages progressed (Sass et al., 1994; Yao et al., 2000). However, in my experiment, emission decreased with an increase in biomass (Fig. 17a,b). Methane emission was highest for plants at the initial tillering stage and lowest for plants at maturity (Fig. 18a). This behavior may have been caused by differences in soil water

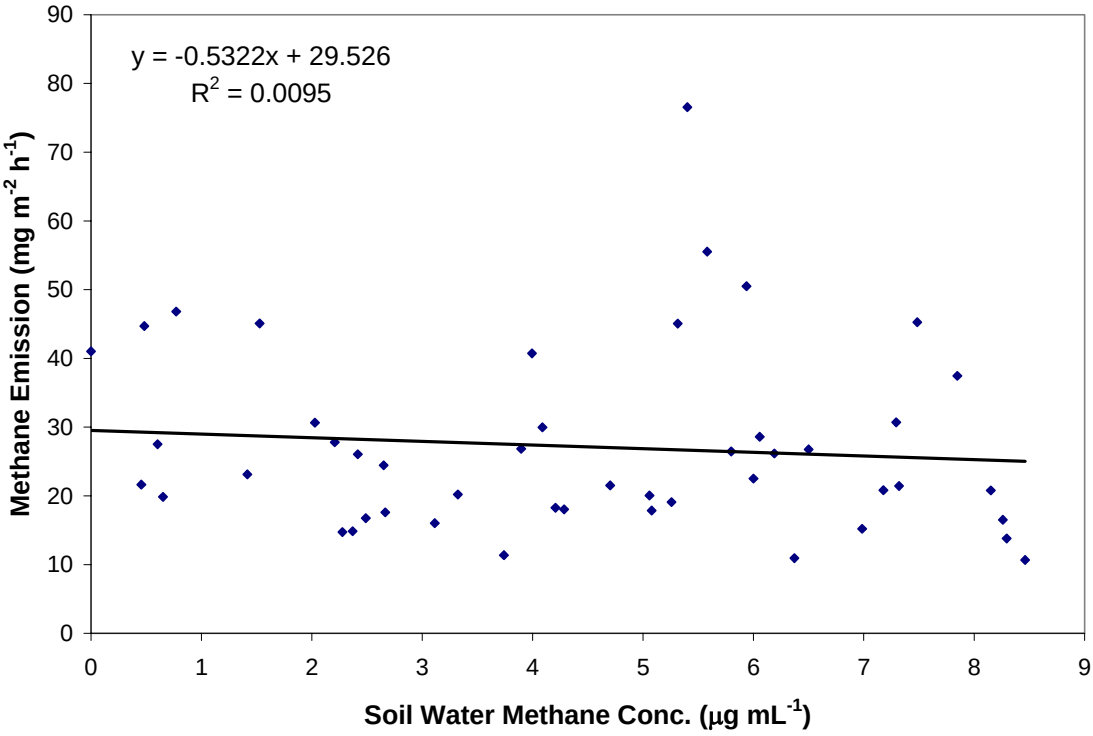

Figure 15. Soil water methane concentration vs soil temperature at 5 cm depth. * denotes significance at the 0.10 level

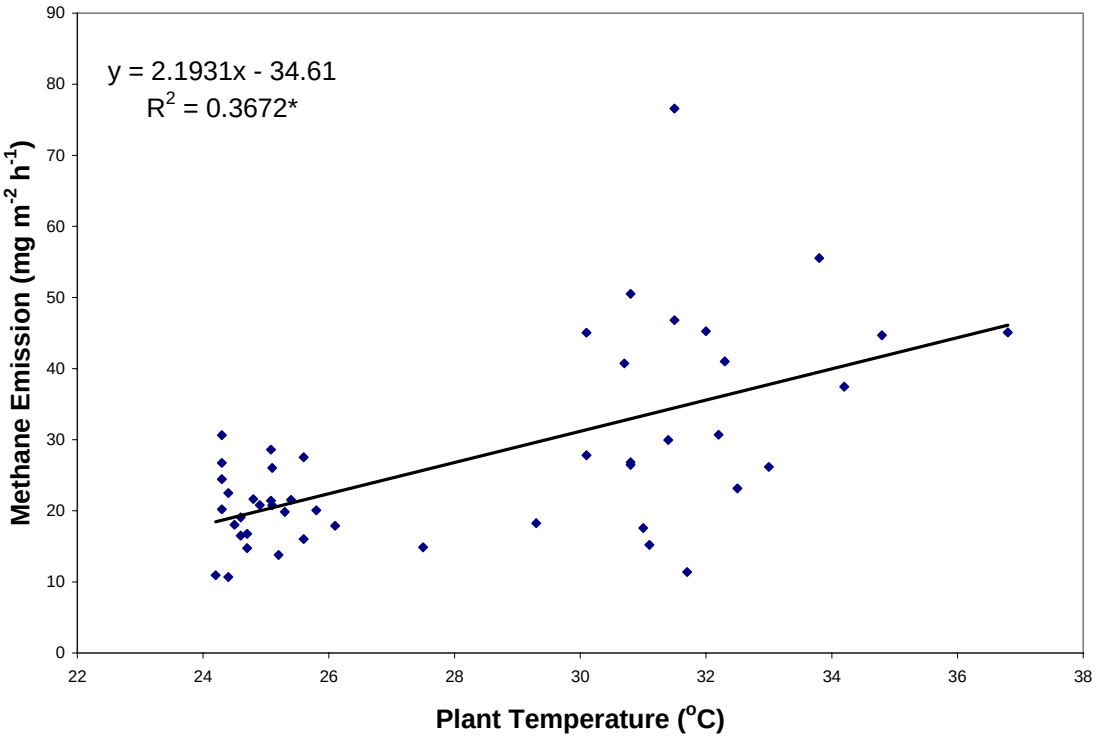

Figure 16. Methane emission vs plant temperature.
*** denotes significance at the 0.10 level**

methane concentration associated with differences in root biomass and activity among plants at different stages of development (Fig. 18b,c). Root biomass and density should have increased as the plant grows and develops but, in the latter growth stages a decrease in root biomass occurred. This could have been caused due to over washing the roots or the decrease in root mass caused by allocation of substrates from the roots to reproductive structures during anthesis and maturity. The root system during maturity experiences a dramatic decrease in size due to the lack of new plant growth and the decomposition of dead plant material.

Possible explanations for the phenomena that I found are: soil rhizosphere oxidation by oxygen diffusion through the roots, decrease in methane conductance with older root tissue, and lack of sufficient organic substrates for methanogenesis.
Root respiration requires oxygen. Rice have aerenchyma that allow oxygen to diffuse from the atmosphere through the plant to the roots. No correlation was found between emission and soil water methane concentration (Fig. 19) however, methane emission, normalized by root biomass, was positively correlated with soil water methane concentration (Fig.20a), and methane concentration was negatively correlated with root biomass (Fig.20b). Much of this oxygen is utilized in root respiration but some is lost by diffusion through the root and into the surrounding rhizosphere resulting in an oxygenated zone around the root (Aulakh et al., 2000). Eventually as root biomass grows and spreads throughout the bucket, the roots oxidize the soil. Methanogens cannot function in oxidized conditions. It is likely that as root biomass increased,

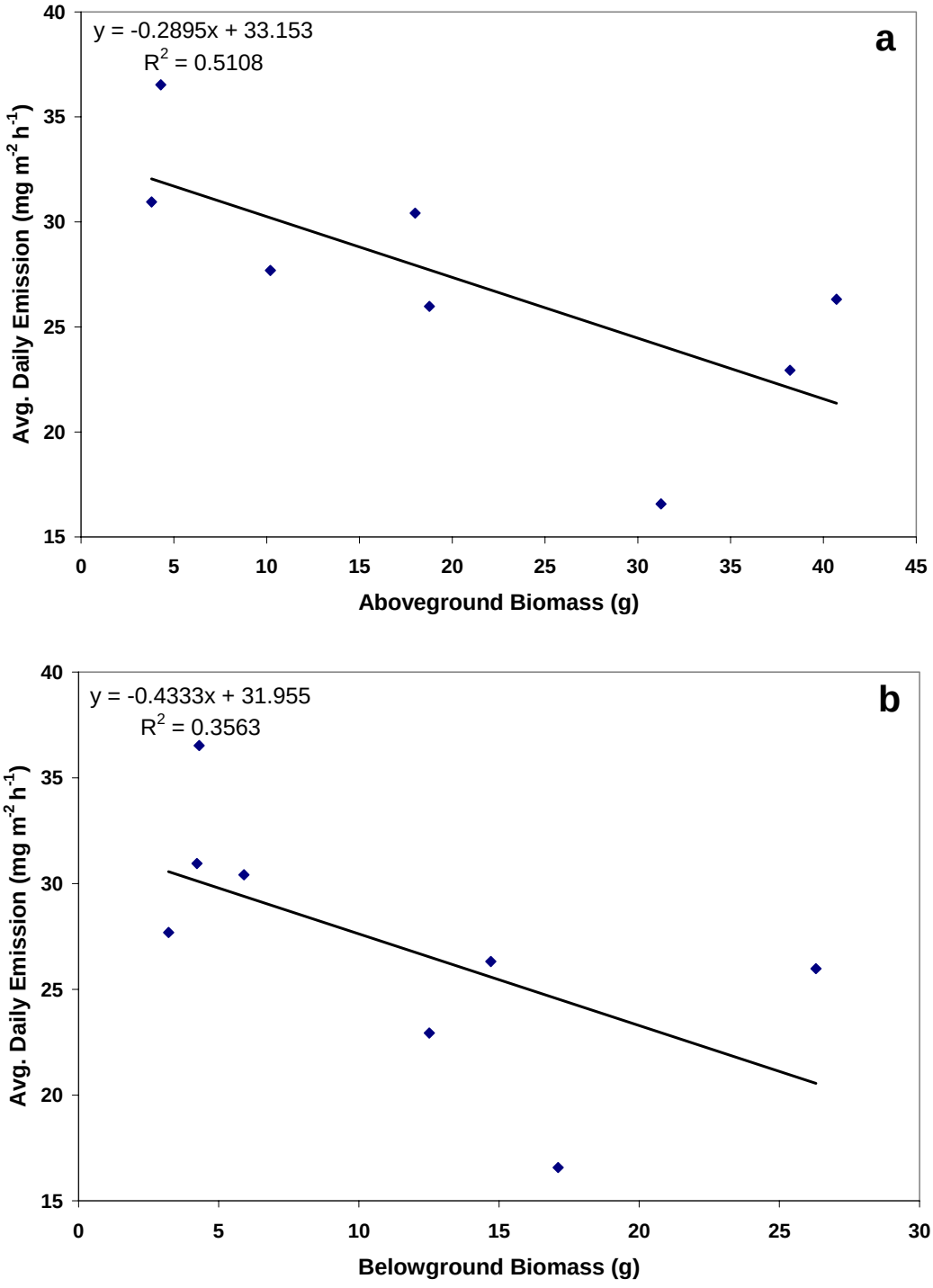

Figure 17. Average hourly emission vs belowground (a) and aboveground (b) biomass.
*** denotes significance at the 0.10 level**

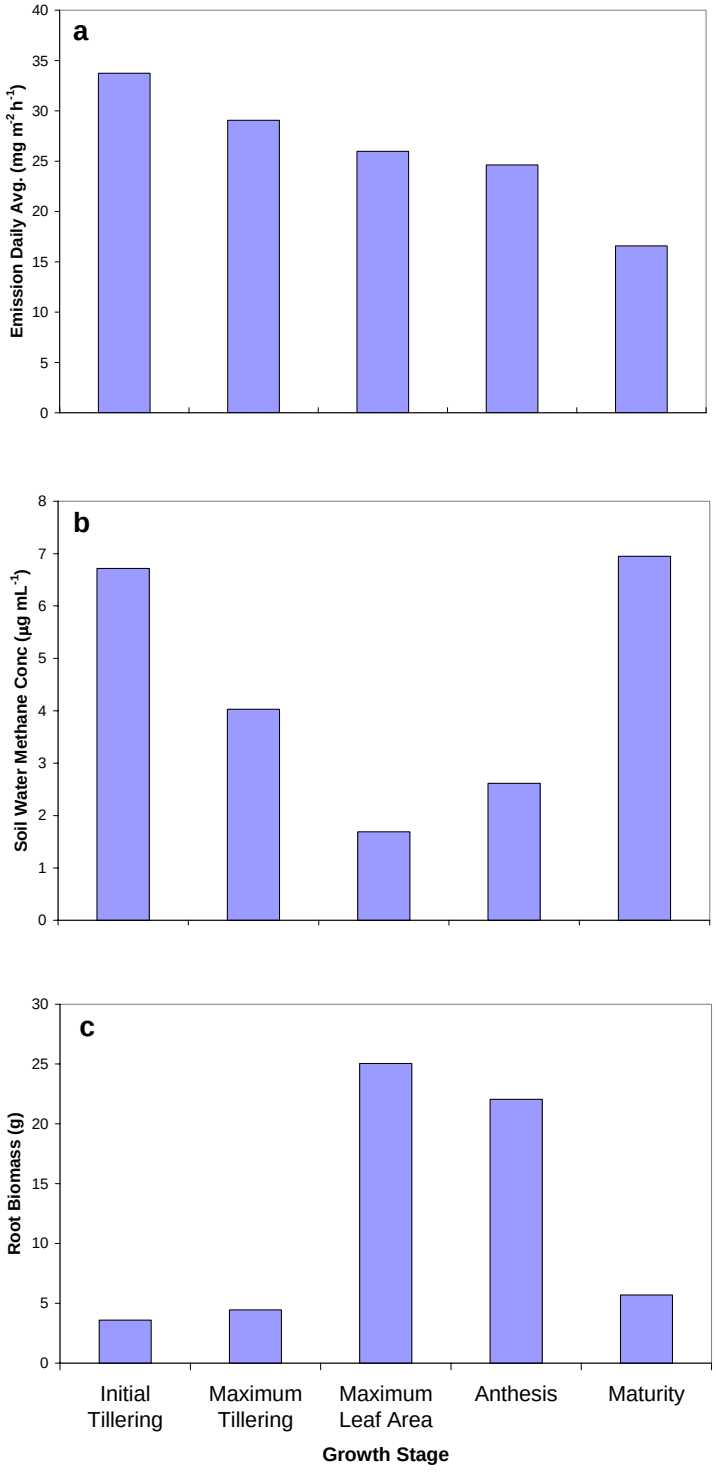

Figure 18. Average hourly methane emission (a), soil water methane concentration (b), and biomass (c) as a function of growth stage

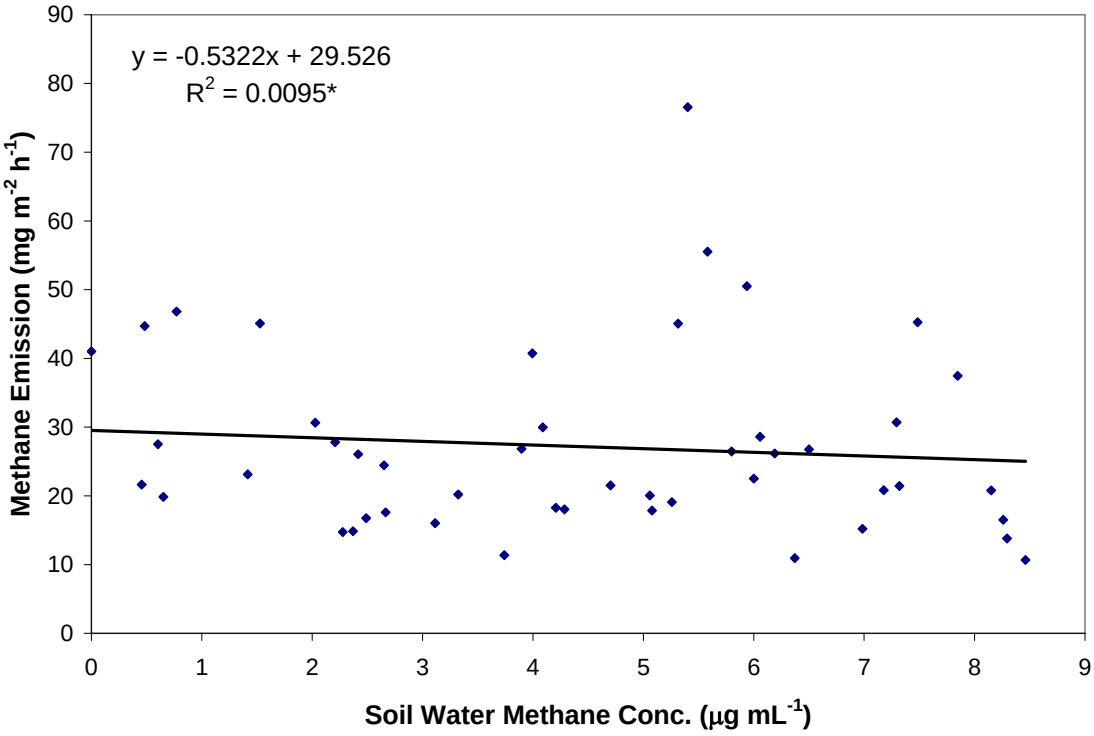

Figure 19. Methane emission vs soil water methane concentration at 5 cm depth. * denotes significance at the 0.10 level

increased oxidation in the root zone decreased the activity of methanogens and created a sink for methane, resulting in a decrease in methane concentration in the soil water and methane emission from plants. It is also likely that root conductance was lower for plants at latter growth stages due to thicker epidermal root cells (Singh et al., 1999). The reduction in methane concentration with time could have been caused by a decrease in organic substrates utilizable by methanogens. The amount of substrates, especially acetic acid, is directly correlated with metabolic activity and methane source strength in the soil (Dunfield et al., 1993, Lindau et al., 1993). In addition the amount of exudates emitted from the roots is decreased when the plant switches from vegetative to reproductive growth phases (Singh et al., 1999).

The decrease in methane emission with growth stage is contrary to what has been found in the literature. The most logical explanation for this decrease in emission with growth stage is that my greenhouse study used buckets that restricted soil volume, creating a closed system. I believe that root density in the soil in my experiment was considerably higher than that of natural systems. Higher densities of root biomass have the potential to greatly oxidize the soil and reduce methane source strength. A highly oxidized soil would occur as a result of a larger root system as the growth stages progressed toward the end of the growth cycle of the plant (Fig. 18c).

During the plants shift from maximum tillering to anthesis, an increase in soil water methane concentration occurred, probably due to either increased root exudates, decreased oxygen diffusion through the roots as a function of greater resistance caused by thicker epidermal root cells and reduced number of viable aerenchyma due to the

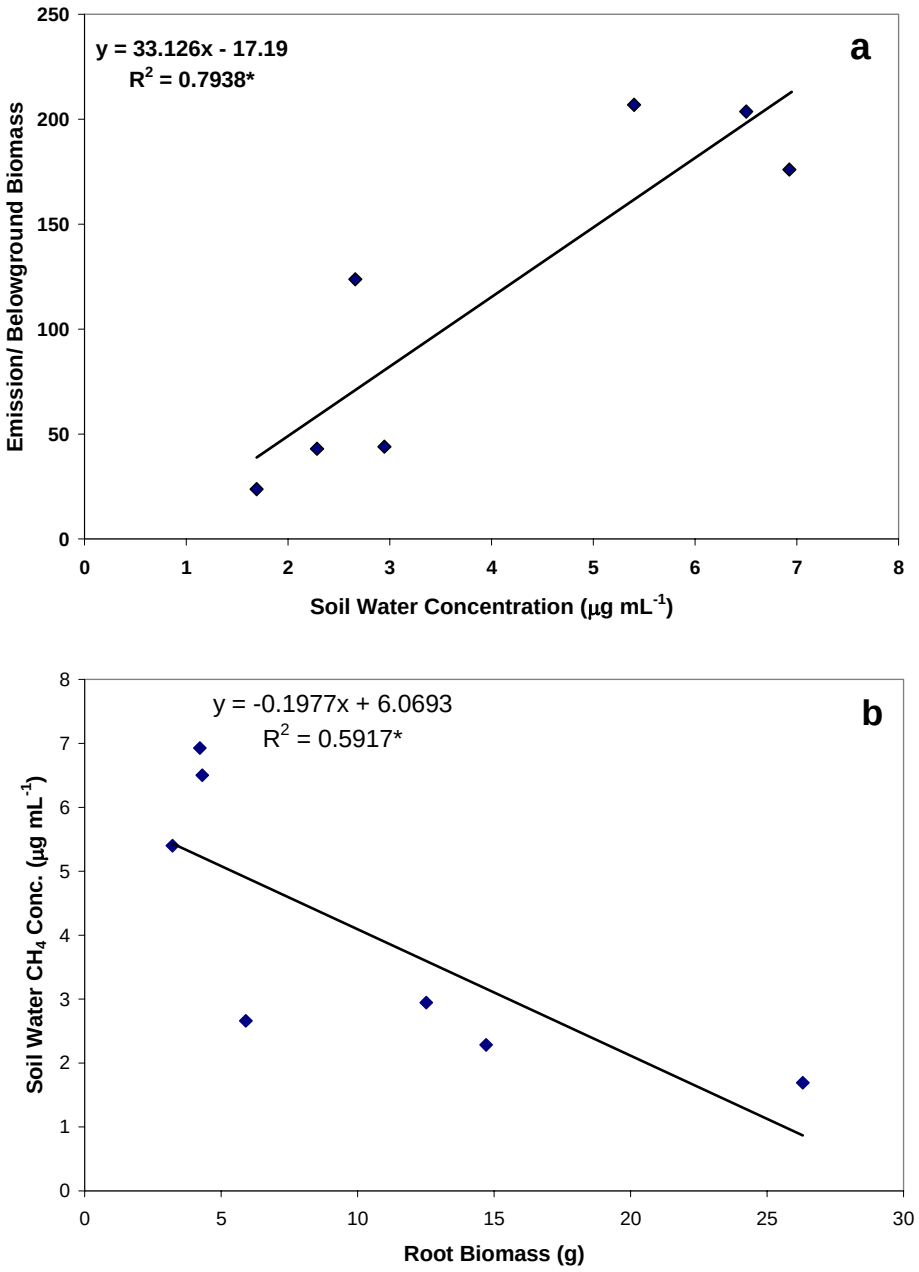

Figure 20. Methane emission/ biomass as a function of soil water methane concentration (a) and soil water methane concentration as a function of root biomass (b).
*** denotes significance at the 0.10 level**

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senescence of tillers during anthesis, decreasing the plants ability for methane conductance and reduced levels of transpiration

SUMMARY AND RECOMMENDATIONS

My research confirmed that diurnal variations in methane emission from rice do occur, and are related impart to diurnal variations in soil temperature. However, plant tissue was shown to be an important factor in methane emission. Soil temperature did not show any correlation with soil water methane concentration but plant temperature positively correlated with emission. Implying that transpiration may act as the driving mechanism for emission through the uptake of water containing methane however, transpiration was not measured and cannot be verified. Emission was highest shortly after solar noon and lowest just prior to sunrise. Methane emission was highest at initial tillering and lowest at maturity, corresponding to differences in soil water methane concentration and root biomass. Increased oxidation of the soil by a larger root biomass in the confined soil volume may have contributed to the increased oxidation status but cannot be confirmed because oxidation status of the soil was not measured.

Improving certain aspects of this project would allow for a clearer result and better understanding of the phenomena of diurnal methane emission in rice plants. Measurement of the reduction-oxidation potential of the soil at various depths would clarify the oxygen status to determine if root rhizosphere oxidation is occurring and at what depth. Measurement of oxygen status could verify that root diffusion of oxygen in to the root rhizosphere is creating an unfavorable environment for methanogens and a favorable environment for methanotrophs. Following specific plants through the growth cycle rather than harvesting plants after measurements would help identify developmental emission patterns, that otherwise would not be noticed using different

plants each time. Measurement of whole plant transpiration and relating that with soil water methane concentration may strongly correlate with emission variation. Measurement of organic matter content of the soil throughout the season would show if organic matter is being utilized and lost from the system, reducing the amount of substrate available for methanogenesis. A reduced amount of available substrates could reduce methane production and methane source strength.

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