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# Influence of soil temperature on methane emission from rice paddy fields

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**Abstract.** Methane emission rates from an Italian rice paddy field showed diel and seasonal variations. The seasonal variations were not closely related to soil temperatures. However, the diel changes of $CH_4$ fluxes were significantly correlated with the diel changes of the temperature in a particular soil depth. The soil depths with the best correlations between $CH_4$ flux and temperature were shallow (1–5 cm) in May and June, deep (10–15 cm) in June and July, and again shallow (1–5 cm) in August. Apparent activation energies ($E_a$) calculated from these correlations using the Arrhenius model were relatively low (50–150 kJ mol$^{-1}$) in May and June, but increased to higher values (80–450 kJ mol$^{-1}$) in August. In the laboratory, $CH_4$ emission from two rice cultures incubated at temperatures between 20 and 38 °C showed $E_a$ values of 41 and 53 kJ mol$^{-1}$. Methane production in anoxic paddy soil suspensions incubated between 7 and 43 °C showed $E_a$ values between 53 and 132 kJ mol$^{-1}$ with an average value of 85 kJ mol$^{-1}$, and in pure cultures of hydrogenotrophic methanogenic bacteria $E_a$ values between 77 and 173 (average 126) kJ mol$^{-1}$. It is suggested that diel changes of soil properties other than temperature affect $CH_4$ emission rates, e.g. diel changes in root exudation or in efficiency of $CH_4$ oxidation in the rhizosphere.

## Introduction

Methane emission rates from rice paddy fields generally show a high temporal variability. Changes of $CH_4$ fluxes with respect to season and daytime have been observed in rice paddy fields in California (Cicerone et al. 1983), in Spain (Seiler et al. 1984), in Italy (Holzapfel-Pschorn & Seiler 1986; Schütz et al. 1989a), and in China (Wang et al. 1990). Temporal variabilities of $CH_4$ emission rates may be due to different effects, e.g. changes in emission pathways of the produced $CH_4$ and in the $CH_4$ oxidation activity (Schütz et al. 1989b); changes in the availability of decomposable organic matter, like stubble, root exudates, root autolysates (Holzapfel-Pschorn et al. 1986; Schütz et al. 1989a); changes in the structure of the methanogenic microbial community (Conrad 1989); changes in soil temperature. The influence of soil temperature is the subject of the present study.

The activities and the growth rates of microorganisms are strongly dependent on temperature. In general, activity and growth rate increase with temperature until an optimal value is reached and then decline rapidly due to inactivation

and killing. Different microbial species can have completely different temperature characteristics. A microbial community basically has the potential to adapt when slow temperature changes allow for selection of the best adapted species, e.g. seasonal temperature changes. This may result in a seasonal succession of temperature-adapted microbes (e.g. Sieburth 1967). On a diel basis, adaptation by succession is unlikely, since it would require very high turnover rates of the microbial populations. In this case, the microorganisms have to respond to temperature changes within the limits of their physiological capacities.

In Californian and Spanish rice paddy fields, $CH_4$ emission rates correlated poorly with seasonal or diel temperature changes (Cicerone et al. 1983; Seiler et al. 1984). In Italian rice paddy fields, on the other hand, $CH_4$ emission rates were found to increase with increasing soil temperature (Holzapfel-Pschorn et al. 1986; Schütz et al. 1989a). This positive correlation was used to explain the sometimes large diurnal variations of $CH_4$ emission rates. However, these reports did not explicitely analyse the relationship between $CH_4$ emission and soil temperature on a seasonal and on a diel basis.

Here we report on the correlation of diel changes of $CH_4$ emission rates with temperature at different soil depths. The data are used to calculate apparent activation energies for $CH_4$ emission and to characterize their seasonal behaviour. The apparent activation energies determined for $CH_4$ emission under *in-situ* conditions are compared to those of $CH_4$ formation in anoxic paddy soil samples and in pure cultures of methanogenic bacteria. The results indicate that the temperature characteristics of the methane formation processes can explain the diel patterns of $CH_4$ emission only in the early season. Factors in addition to the diel change of soil temperatures are required to explain the sometimes high diel amplitudes of $CH_4$ emission in the summer and autumn.

## Methods

### *Field measurements*

Field measurements of $CH_4$ emission rates were made in rice paddy fields of the Italian Rice Research Institute near Vercelli, in the valley of the river Po. The field site and soil characteristics have already been described (Schütz et al. 1989a, b). Methane emission rates were measured by the static box techniques using the fully automated, computerized sampling and analysis system described in detail by Schütz et al. (1989a). The system allowed the determination of $CH_4$ emission rates at 16 individual field plots once every 3 hours, thus obtaining 8 flux rates per plot per day. The measurements were performed in 1984 through 1986 giving a three years continuous record which has been presented and discussed by Schütz et al. (1989a). These data of $CH_4$ emission were used in this paper.

Soil temperatures in the paddy soil were recorded continuously at one of the

field plots using Pt-100 thermocouples at depths of 1, 5, 10, 15 and 23 cm. Sporadic measurements of the soil temperature (1 cm and 5 cm depth) at other field plots showed comparable values.

*Laboratory measurements*

The temperature dependence of $CH_4$ emission was determined in rice cultures which were prepared as described by Holzapfel-Pschorn et al. (1985, 1986). Glass beakers (800 ml volume) were filled with 600 g d.w. of air-dried paddy soil and flooded with distilled water. Each beaker was planted with 10 pregerminated rice seeds, incubated in a water bath at 20 °C and illuminated with incandescent light in a radiation intensity of ca. 200 $W m^{-2}$. The light regime was kept at a cycle of 13–14 h light and 10–11 h dark. $CH_4$ emission rates were measured by placing the beakers with the rice cultures in a large incubation vessel described earlier (Holzapfel-Pschorn et al. 1985) and measuring the increase of $CH_4$ in the atmosphere of this vessel. After about 1 month of incubation the rice plants had developed and $CH_4$ emission rates were relatively constant at about 10 mg $CH_4$ $m^{-2}$ $h^{-1}$ at 20 °C. The incubation temperature of the rice cultures was then increased. After one day of temperature adaptation the rate of $CH_4$ emission was measured again. Two sets of experiments were conducted: one rice culture measured at temperatures of 21, 25, 30 and 36 °C ($n$ = 4), and 3 replicate rice cultures measured at 6 temperatures between 20 and 38 °C ($n$ = 18).

The temperature dependence of $CH_4$ formation was measured in slurries of anoxic Italian paddy soil prepared as described by Conrad et al. (1987). The soil slurries (10 g d.w. soil and 10 ml $H_2O$) were incubated at 25 °C in serum bottles (120 ml) under an atmosphere of $N_2$ until $CH_4$ production rates reached a relatively constant value (after 4–10 days). Then the gas atmosphere was exchanged with $N_2$ or $N_2$:$CO_2$ (8:2), and incubation continued for about 5–10 days. In some experiments, acetate (5 mM) was added as substrate to the slurries, in others $H_2$ : $CO_2$ (8 : 2) was used as atmosphere. After this preincubation, the gas atmosphere was again exchanged and the bottles were acclimated overnight (most in triplicate) to 7, 15, 20, 25, 30, 35, and 43 °C. After temperature acclimation, the rate of $CH_4$ production at the particular temperature was determined from the linear increase of the $CH_4$ mixing ratio over 24 h. In a few experiments, methanogenic substrates ($H_2$ or acetate) were added to soil slurries which had been acclimated to the temperatures for 1–2 weeks. After addition of substrate and exchange of the gas phase the increase of $CH_4$ was measured for 2–4 days with the $CH_4$ production rates being determined each day.

Similar experiments were conducted with pure cultures of methanogenic bacteria. *Methanobrevibacter arboriphilus* (DSM 744), *Methanobacterium bryantii* (DSM 863) and *Methanospirillum hungatei* (DSM 864) were obtained from the German Collection of Microorganisms (DSM). *Mb. bryantii* strain Babl has been isolated from the paddy soil (Conrad et al. 1989a). The bacteria

were grown at 30 °C under a $H_2$:$CO_2$ (8:2) atmosphere in the carbonate-buffered (pH 7), sulfide-reduced mineral medium described by Widdel & Pfennig (1981). Acetate (2 mM) was added as an additional carbon source. Cultures at the end of exponential growth phase were distributed into bottles with fresh medium and $H_2$:$CO_2$ atmosphere, and acclimated overnight to the assay temperatures of 7–43 °C. Then, the bottles were flushed with $H_2$:$CO_2$ (8:2), and the $CH_4$ production rates were determined from the linear increase of the $CH_4$ mixing ratio in the headspace. The density of the bacterial cultures was determined by measuring the extinction at 578 nm in a photometer.

*Data analysis*

Methane emission and $CH_4$ production rates were correlated with temperature using the integrated form of the Arrhenius equation:

$$\ln \text{Rate} = (1/T)(-E_a/R) + \ln A \quad (1)$$

where $A$ = Arrhenius constant, $E_a$ = apparent activation energy; $R$ = gas constant; $T$ = temperature (K). The use of the Arrhenius model is an empirical approach. The calculated apparent activation energy is not an activation energy in the chemical sense, rather it is a measure of the temperature characteristic of the $CH_4$-producing system.

## Results

*Field experiments*

The measurements were performed on two field plots in 1985. One of the plots was untreated, the other had been fertilized with rice straw which was incorporated into the soil prior to flooding. This plot thus had a higher content of organic matter which can be decomposed to $CH_4$ and consequently, showed significantly higher $CH_4$ emission rates than the untreated field plot (Fig. 1).

Similar, but somewhat less complete data sets exist for the 1984 and 1986 growing seasons. The analysis of these data sets gave results comparable to those of 1985. In the following, however, we concentrate on the data from 1985.

Methane emission rates as well as soil temperatures showed a significant seasonal pattern. The seasonal change of temperature at 5 cm depth and the rates of $CH_4$ emission in two different field plots are shown in Fig. 1. These data indicate that there was not a close correlation between soil temperature and $CH_4$ fluxes on a seasonal basis, although the general trends of both were similar especially in the straw-fertilized plot. Similar seasonal trends were described before (Holzapfel-Pschorn & Seiler 1986; Schütz et al. 1989a).

The bars of the data in Fig. 1 represent the amplitudes of the diel variations of soil temperatures as well as of $CH_4$ fluxes. Again, there is no evidence for a

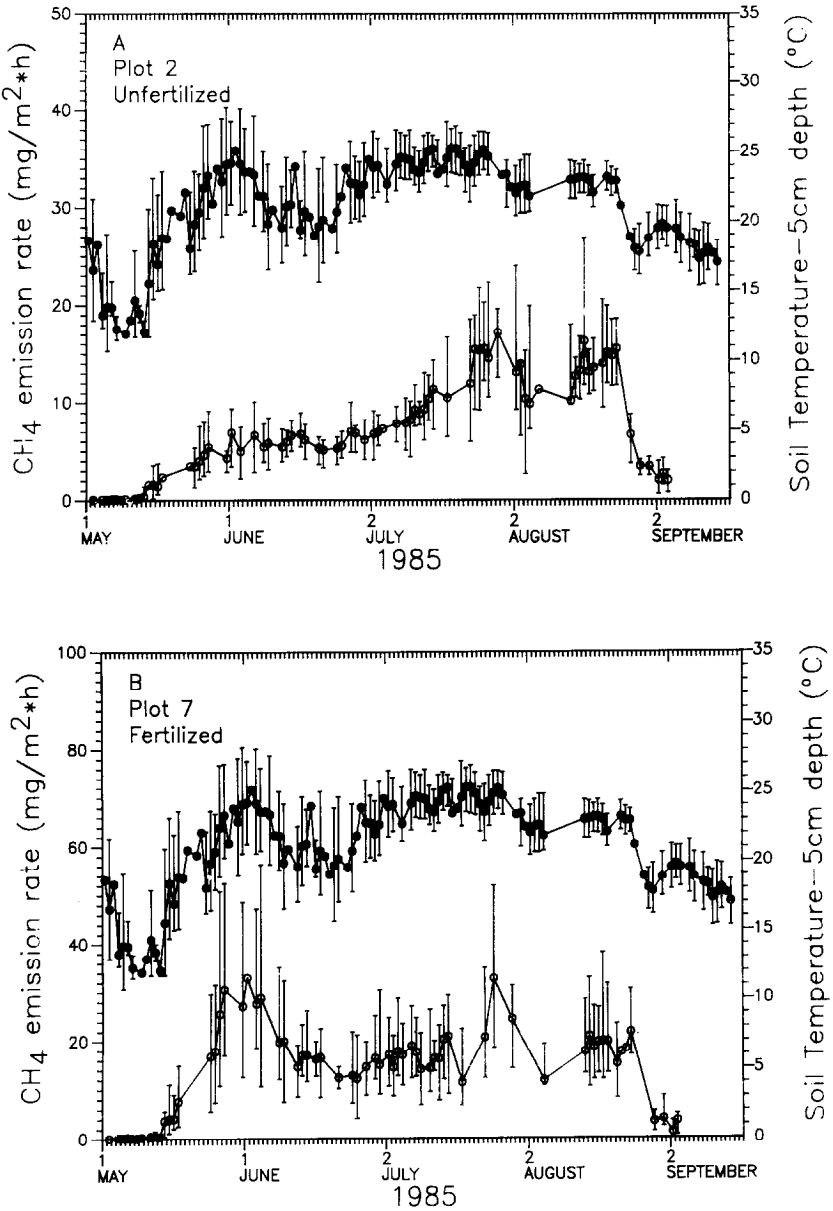


*Fig. 1.* Seasonal variations of the daily averages and ranges of $CH_4$ emission rates (○) and soil temperatures (●; 5 cm depth) in two different rice paddies. One of the paddies (plot 2) was untreated (*A*) and the other (plot 7) was fertilized with 12 tons of rice straw per ha (*B*).

general correlation of high temperature amplitudes with high flux amplitudes. Temperature amplitudes generally decreased during the season due to shading of the soil surface by the developing rice plants. Amplitudes of $CH_4$ fluxes, on the other hand, were high in spring and fall, but smaller in between.

A
B
a
b
CH4 emission rate (mg/m2*h)
Soil Temperature (°C)
Date/Time (hh:mm)
26 MAY
27 MAY
16 JUNE
17 JUNE

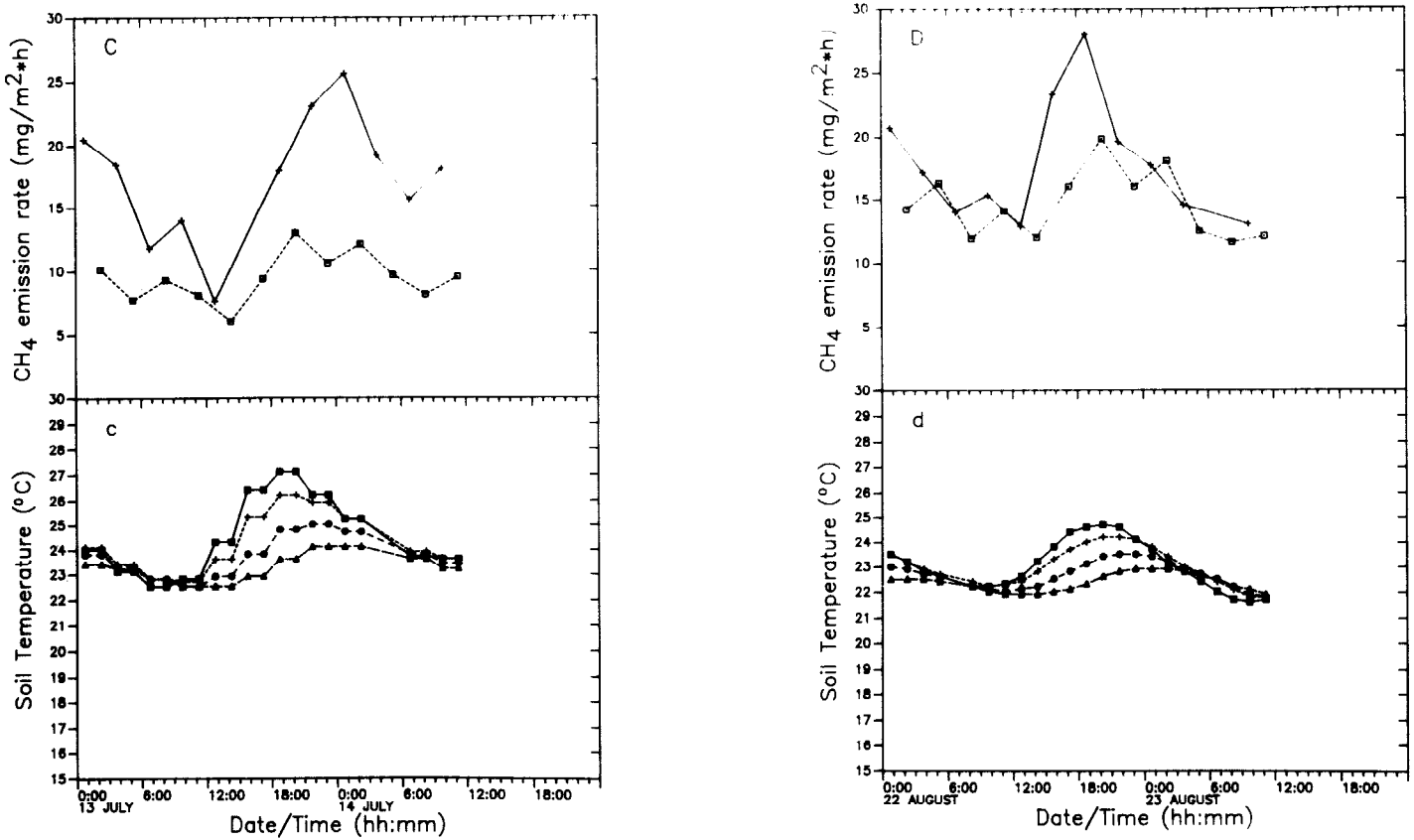


*Fig. 2.* Diel changes of $CH_4$ emission rates (A, B, C, D) in the untreated (□) and in the straw-fertilized rice paddy (+), as well as diel changes of soil temperatures (a, b, c, d) measured at 1 cm (□), 5 cm (+), 10 cm (○), and 15 cm (△) depth. The data were obtained in May, 26/27 (A, a), June, 16/17 (B, b), July, 13/14 (C, c), and August, 22/23, 1985 (D, d).

These relationships were further analysed by comparing the diel change in $CH_4$ flux with that of soil temperatures measured at different depths. For example, the diel changes in $CH_4$ flux and soil temperature at 1, 5, 10, and 15 cm depth are shown in Fig. 2A-D for May through August, 1985, respectively. As expected, the diel amplitudes of soil temperatures decreased with increasing depth. In addition, at the depth the maximum temperature was reached increasingly later during the day. The temperature maxima were observed between 17.00 and 2.00 local time depending on the date and the soil depth. The $CH_4$ fluxes peaked in the same time period as the soil temperatures indicating that they were influenced by the soil temperature. However, the $CH_4$ fluxes of the different plots and on the different dates correlated best with temperatures in different depths (Table 1). Correlations were calculated with the Arrhenius model. The data from June, 16 (Fig. 2B, b) did not show a significant correlation ($P < 95\%$) between $CH_4$ flux and soil temperature. The data from the other months, however, resulted in correlation coefficients ($r$) of 0.86 to 0.93 which are significant for $n = 8$ data pairs at the 99% level. For example, $CH_4$ fluxes of the straw-fertilized plot correlated best with soil temperatures at 1 cm, 15 cm, and 1 cm depth in May, July, and August, respectively (Table 1).

The correlation coefficients for $CH_4$ flux vs soil temperatures at different depths are summarized in Fig. 3 for the individual days in 1985 using the straw-fertilized field plot. For most of the dates $CH_4$ fluxes and soil temperatures showed correlation coefficients of $r > 0.79$ or $r > 0.62$ which are significant for $n = 8$ data pairs at the 99% or 95% level, respectively. The data also show that the soil depths whose temperatures correlated best with the observed $CH_4$ emission rates frequently changed during the course of the rice growing season. As a trend, temperatures in 10 and 15 cm depth showed best correlations in June and July, whereas temperatures in 1 and 5 cm depths were better in May and August.

The apparent activation energies calculated from the correlations of $CH_4$ flux vs soil temperature are summarized in Fig. 4A, B for the untreated and the straw-fertilized field plots and for the 1 and 15 cm depths. As expected, $E_a$ values were much higher at 15 cm than at 1 cm depth because the same range of $CH_4$ fluxes had to be correlated to the relatively small temperature amplitudes at 15 cm depth. Interestingly, the values of $E_a$ were smaller in June than in August, when the rice plants had already shooted. Especially in the straw-fertilized plot, apparent activation energies of > 200 kJ per mole $CH_4$ were calculated. These high values were obtained even if the diel $CH_4$ fluxes were correlated to the soil temperature with the highest amplitudes, i.e. in 1 cm depth. Correlations to 15 cm depth resulted in unrealistically high values of $E_a > 450$ kJ.

Assuming that realistic apparent activation energies are calculated from temperatures in that depth which gives the best correlation, an optimized seasonal distribution of $E_a$-values together with the corresponding soil depths was obtained (data not shown). The results indicate a general trend of best correlations changing from shallow, to deep, and again to shallow soil temperatures during the course of the season. The thus calculated $E_a$ values generally were based on highly significant correlations ($P > 99\%$). They ranged

*Table 1.* Apparent activation energies ($E_a$) and correlation of $CH_4$ emission rates with temperature at different soil depths.[1]

| Date | Field plot | $E_a$ (kJ mol$^{-1}$) | Soil depth (cm) | r |
|---|---|---|---|---|
| May 26 | 2 | **46** | **1** | **0.83** |
| | | 50 | 5 | 0.80 |
| | | 56 | 10 | 0.73 |
| | | 56 | 15 | 0.61* |
| | 7 | **108** | **1** | **0.93** |
| | | 122 | 5 | 0.91 |
| | | 143 | 10 | 0.85 |
| | | 155 | 15 | 0.72 |
| June 16 | 2 | 59 | 1 | 0.49* |
| | | 43 | 5 | 0.45* |
| | | 24 | 10 | 0.39* |
| | | 11 | 15 | 0.29* |
| | 7 | 56 | 1 | 0.61* |
| | | 28 | 5 | 0.45* |
| | | 2 | 10 | 0.15* |
| | | −10 | 15 | −0.31* |
| July 13 | 2 | 55 | 1 | 0.76 |
| | | 83 | 5 | 0.82 |
| | | **140** | **10** | **0.87** |
| | | 214 | 15 | 0.85 |
| | 7 | 62 | 1 | 0.62 |
| | | 108 | 5 | 0.74 |
| | | 200 | 10 | 0.84 |
| | | **385** | **15** | **0.91** |
| August 22 | 2 | 90 | 1 | 0.83 |
| | | **125** | **5** | **0.87** |
| | | 172 | 10 | 0.87 |
| | | 222 | 15 | 0.79 |
| | 7 | **133** | **1** | **0.86** |
| | | 155 | 5 | 0.84 |
| | | 171 | 10 | 0.77 |
| | | 185 | 15 | 0.65 |

[1] The values were calculated from the data shown in Fig. 2. The field plots were untreated (#2) and fertilized with rice straaw (#7), respectively. Correlations were significant at $P > 95\%$ except those marked with a star. The best correlations for each day and plot are in bold face.

between 40 and 440 kJ mol$^{-1}$ similar as those (Fig. 4) which had not been optimized.

When the $E_a$-values were plotted against the average value of the corresponding $CH_4$ fluxes observed on the same day, the data pairs from the entire season resulted in a highly scattered picture which did not give a significant correlation (data not shown). However, in May alone there was a decreasing trend of the apparent activation energies with increasing $CH_4$ emission rates.

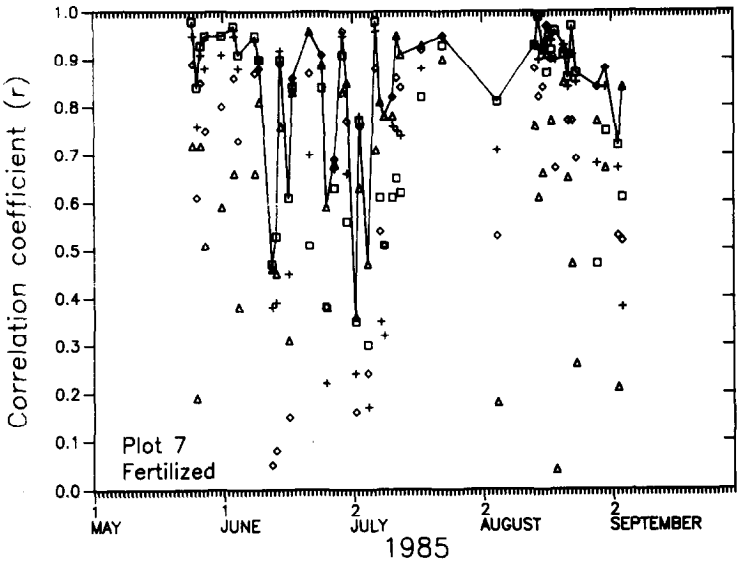


*Fig. 3.* Seasonal variation of the correlation coefficients ($r$) obtained for the Arrhenius correlation between $CH_4$ emission rates and soil temperatures measured in 1 cm (□), 5 cm (+), 10 cm (◇), and 15 cm (△) depth in the straw-fertilized rice paddy. The line connects the highest correlation coefficient determined for each day.

*Laboratory experiments*

The rates of $CH_4$ emission increased when rice cultures (pots with soil plus rice plants) were incubated at increasing temperatures. The laboratory studies simulated field conditions with respect to submergence, vegetation, and exposure to an air atmosphere, but did not simulate field conditions with decreasing soil temperature with increasing soil depth. Two different sets of experiments gave the following results:

$n = 4$; 21–36 °C; $E_a = 41\,\text{kJ mol}^{-1}$; $r = 0.98$; and

$n = 18$; 20–38 °C; $E_a = 53\,\text{kJ mol}^{-1}$; $r = 0.95$.

Incubation of slurries of anoxic paddy soil under a $N_2$ or $N_2/CO_2$ atmosphere resulted in $CH_4$ formation rates at 25 °C of about 20–35 $\text{nmol h}^{-1}\,\text{g}^{-1}$ d.w. (compare Conrad et al. 1987). The rates steadily increased with temperature, but decreased above 40 °C due to inactivation of the microorganisms. Apparent activation energies were calculated from the rates measured over a temperature range from 7 to 35 °C using the Arrhenius model. The correlation between $CH_4$ formation rates and incubation temperatures were significant at the 99% level.

The apparent activation energies obtained from the various experiments are summarized together with the $CH_4$ production rates at 25 °C in Fig. 5. The individual data points are averages from triplicate experiments which were precise within limits of 3–19%. The values from the different experiments ranged between 53 and 132 $\text{kJ mol}^{-1}$ $CH_4$, with most frequent values between 80

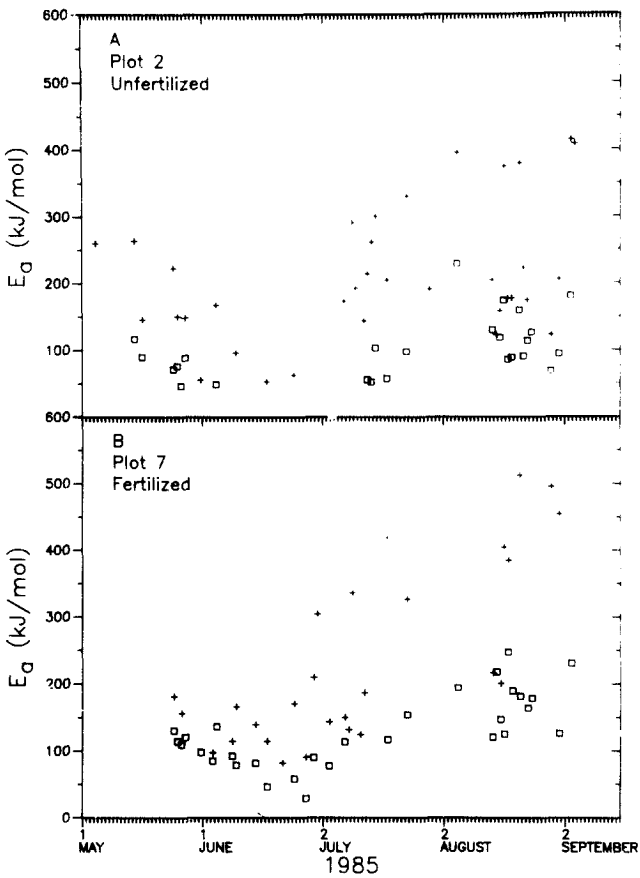


*Fig. 4.* Seasonal variations of the apparent activation energies ($E_a$) for $CH_4$ emission calculated for soil temperatures measured at 1 cm (□) or at 15 cm (+) depth in the untreated (*A*) and the straw-fertilized (*B*) rice paddy.

and 90 kJ $mol^{-1}$ $CH_4$, and an average value of 85 kJ $mol^{-1}$ $CH_4$. Slurries which had been preincubated with methanogenic substrates, such as $H_2$ or acetate, showed higher $CH_4$ production rates at 25 °C than those without substrates. The $E_a$ values, however, were only slightly higher after preincubation with substrates (Fig. 5).

Immediately after $H_2$ was added as methanogenic substrate $CH_4$ production rates increased from day to day (3 experiments; Fig. 5). This increase was probably due to an increase of the size of the methanogenic population. During this phase there was a significant trend towards increasing $E_a$ values. The presence of $H_2$ apparently stimulated a temporal increase of $CH_4$ production rates especially at high temperatures (compare Conrad et al. 1987). In this phase, there was a positive correlation between the rate of $CH_4$ production and the $E_a$-value (Fig. 5). Experiments with addition of acetate or controls without

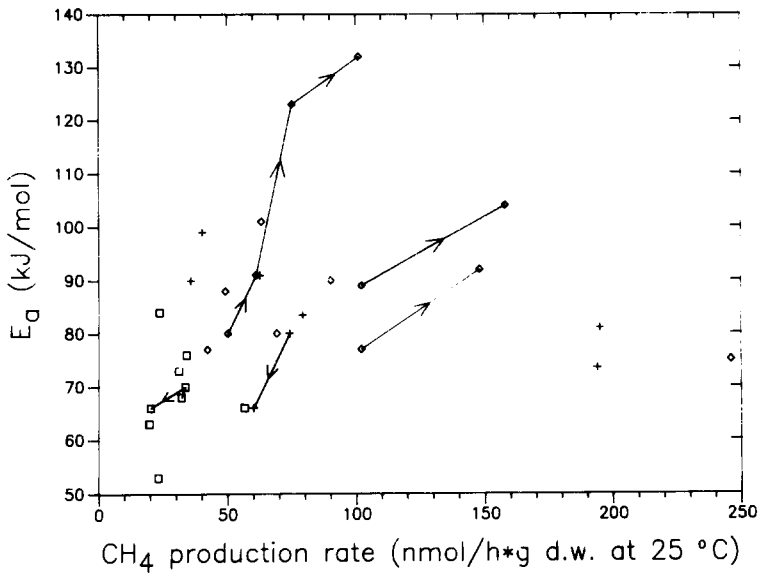


*Fig. 5.* Apparent activation energies ($E_a$) against $CH_4$ production rates at 25 °C in slurries of anaerobically incubated rice paddy soil. The slurries had been prepared without additional substrate (□), with 5 mM acetate (+), or under a $H_2/CO_2$ (8:2) atmosphere (◇), acclimated overnight to different temperatures and then assayed at these temperatures for 24 h. Lines with arrows mark the change in $E_a$ and $CH_4$ production 1–4 days after substrate addition to temperature-acclimated soil slurries (see text).

substrate addition (1 experiment each; Fig. 5) only showed slight changes in $CH_4$ production and in $E_a$ after 1–2 days of extended incubation.

Methane production also was measured in pure cultures of hydrogenotrophic methanogens incubated at different temperatures. After temperature acclimation overnight, the bacterial density had increased about 2 fold at the high, but had not changed at 5–10 °C. However, during the subsequent measurement of the $CH_4$ production rate (typically lasting 6 hours) $CH_4$ increased linearly and the value of $E_{578}$ did not change significantly. The measured $CH_4$ production rates were normalized to $E_{578} = 0.10$ before they were used to calculate the apparent activation energies according to the Arrhenius model.

Table 2 summarizes the measured rates of $CH_4$ production normalized to $E_{578} = 0.1$ and the apparent activation energies determined for a temperature range of 20 to 35 °C. The minimum temperatures with detectable $CH_4$ formation were about 15 °C (5 °C for *Mbr. arboriphilus*), the optimum temperatures were generally around 35 °C, and the maximum temperatures were higher than 40 °C. The $E_a$-values ranged between 77 and 173 kJ $mol^{-1}$ $CH_4$ with an average value of 126 kJ $mol^{-1}$ $CH_4$. The $E_a$ value for *Msp. hungatei* in an exponentially growing culture was almost double of that in a culture which had already reached the stationary phase. Although the data base is very small, it indicates a positive correlation between $CH_4$ production and apparent activation energy.

*Table 2.* Temperature characteristics of $CH_4$ production by different strains of methanogenic bacteria.[1]

| Strain | Age of culture (days) | Activity at 25°C (nmol $h^{-1}$ $ml^{-1}$) | $E_a$ (kJ $mol^{-1}$) |
|---|---|---|---|
| *Msp. hungatei* | 13 | 87 | 94 |
| *Msp. hungatei* | 6 | 312 | 173 |
| *Mbr. arboriphilus* | 8 | 254 | 77 |
| *Mb. bryantii* | 7 | 123 | 116 |
| *Mb. bryantii Bab1* | 6 | 360 | 170 |

[1] The bacteria were cultivated at 28°C in mineral medium supplemented with 2 mM acetate under a $H_2/CO_2$ (8:2) atmosphere for the time indicated. The rates of $CH_4$ production were normalized to 1 ml of cell suspension with a turbidity of $E_{578} = 0.1$.

## Discussion

### *Seasonal change of flux-temperature relations*

Biological activities in general are temperature-dependent. Hence, it is not unexpected that the diel changes of $CH_4$ emission rates from an Italian rice paddy field were positively related to the diel changes in soil temperature. For most days of the growing season, correlations were significant at the 99% or at least at the 95% probability level (Fig. 3).

The diel rhythm of soil temperature changed with soil depth and showed an increasing phase shift to later daytimes probably because of the time-dependent heat transport into deeper soil. In addition, the amplitudes of the diel temperature changes decreased with soil depth. In the Italian paddy soil the amplitudes were less than 0.5 °C below 20 cm so that $CH_4$-emitting processes must have been localized in shallower depths, if the diel changes in $CH_4$ flux were to be due to changes in soil temperature. The Arrhenius model made it possible to determine the soil depth where the diel rhythm of temperature and $CH_4$ flux showed the best coincidence. The results indicate that the soil depths with the best correlation of temperature to $CH_4$ emission were shallow (1–5 cm) in May and June, deep (10–15 cm) in June and July, and shallow (1–5 cm) in August.

One can argue that the soil depth with the best correlation between temperature and $CH_4$ flux would be the depth where the processes are predominantly localized that ultimately cause the $CH_4$ emission into the atmosphere. We assume that in May and early June $CH_4$ was produced from soil organic matter throughout the soil profile, and that $CH_4$ production was stimulated by temperature especially in shallow soil depths. In July and August, on the other hand, the predominant $CH_4$-emitting processes probably were localized within the rhizosphere and thus, $CH_4$ emission was dependent on the temperature regime at this soil depth. Since diel temperature amplitudes were only small in this depth, the diel changes in $CH_4$ emission were probably caused by other environmental variables (see below). This argument is consistent with earlier observations on tillering of rice plants and on transition from $CH_4$ emission via

ebullition to $CH_4$ emission via vascular transport (Holzapfel-Pschorn and Seiler 1986; Schütz et al. 1989a, b).

*Apparent activation energies of $CH_4$ flux*

The apparent activation energy ($E_a$) comprises the temperature response of the $CH_4$-producing microbial community, including polymer-hydrolyzing, fermenting, and methanogenic bacteria; it also comprises the temperature response of the $CH_4$-oxidizing microbial community present in the surface layers of the paddy soil and at the surface of the rice roots (DeBont et al. 1978; Schütz et al. 1989b); and finally comprises the temperature response of the physical transport of $CH_4$ into the atmosphere. The apparent activation energy of $CH_4$ flux is thus a measure of the temperature response of the overall reaction.

In addition, it must be emphasized that the $CH_4$ flux integrates processes occurring throughout the soil profile, regardless of their localization, while the temperature is recorded in a specified soil depth. Hence, the correlation of $CH_4$ flux to the temperature at a slightly shallower soil layer with higher temperature amplitudes would result in lower values of $E_a$ than correlation of the same flux to the temperature at deeper soil layers and vice versa. Calculations in fact showed this response of $E_a$ (Figure 4). By using the temperatures of the soil depth that gives the best possible correlation to the $CH_4$ flux we calculated apparent activation energies changing between 40 and 440 kJ mol$^{-1}$ $CH_4$ emitted. One feature of the seasonal change of apparent activation energies for $CH_4$ emission was especially pronounced: the relatively low $E_a$-values in May and June and the relatively high $E_a$-values in July and August.

In May and early June, $E_a$ values of $CH_4$ emission were less than 150 kJ mol$^{-1}$ $CH_4$ and were similar to $E_a$ values measured for $CH_4$ production in anoxic soil and in pure cultures of methanogens. Within the same range are other values reported for laboratory measurements of $CH_4$ production, e.g. by the acetotrophic *Methanothrix soehngenii* (157 kJ mol$^{-1}$ $CH_4$; Gujer & Zehnder 1983), by samples of Italian paddy soil (68–90 kJ mol$^{-1}$; Conrad et al. 1987), Japanese paddy soil (90 kJ mol$^{-1}$; Koyama 1963), lake sediment (25–180 kJ mol$^{-1}$; Kelly & Chynoweth 1981), peat (117–127 kJ mol$^{-1}$; Svensson & Rosswall 1984), or alder swamp sediment (92–110 kJ mol$^{-1}$; Westermann & Ahring 1987).

In May, the microbial communities adapt to the transition from dry oxic to submerged anoxic soil conditions. Thus, the ecosystem is not yet influenced by the rice plants and $CH_4$ is largely emitted by ebullition (Schütz et al. 1989b). In contrast to those in August, $E_a$ values in May showed a significant negative correlation with the average daily $CH_4$ emission rates. A similar behaviour was reported for sulfate reduction in marine sediments by Westrich & Berner (1988). These authors suggested that recalcitrant organic matter might have a higher activation energy for decomposition thus explaining the observed negative correlation.

*Diel flux changes due to indirect temperature effects*

$E_a$ values in July and August were significantly higher than the values measured in laboratory experiments. Large diel changes of $CH_4$ flux apparently persisted although changes in soil temperature were small. This situation cannot be explained by a direct temperature activation of the methane-producing microbial activity, but may be due to the operation of additional processes enhancing the diel temperature effect, e.g. 1. growth of methanogenic bacteria, 2. activity of microbes producing methanogenic substrates, 3. microbial community changes, and 4. oxidation of methane.

*Growth of methanogenic bacteria*

Heat not only stimulates bacterial activity, but may also stimulate growth or enzyme synthesis resulting in additional $CH_4$ production. However, reports on temperature-dependent growth of *Mtx. soenhgenii* (Gujer & Zehnder 1983) and *Mb. bryantii* (Conrad et al. 1989a) indicate much lower $E_a$-values for cell multiplication (36–43 kJ $mol^{-1}$ bacteria) than for $CH_4$ production (157–170 kJ $mol^{-1}$). Hence, growth or enzyme synthesis is an unlikely explanation for the high $E_a$-values observed during July and August. Furthermore, $E_a$ values for $CH_4$ emission were determined on a day to day basis and any effect by temperature-stimulated bacterial growth must have occured at the same frequency. This would require a relatively high turnover of bacterial biomass. Turnover rates of bacterial biomass in paddy soil are unknown. In sediments, bacterial growth rates may reach values of $10^6$–$10^8$ cells $g^{-1}$ d.w. $day^{-1}$ (Moriarty 1986; Thorn & Ventullo 1988). Using this value and a microbial biomass of 1–7 $\times$ $10^{10}$ cells $g^{-1}$ d.w. as determined for Japanese paddy soils (Hasebe et al. 1984), the daily bacterial turnover would be less than 1% of the total bacterial biomass. Hence, it is not very likely that bacterial growth would have an influence on the temperature response of $CH_4$ emission. The population of acetotrophic and hydrogenotrophic methanogens did also not significantly change during the growing season and thus cannot explain the seasonal changes in $CH_4$ emission (Schütz et al. 1989b).

*Activity of microbes producing methanogenic substrates*

It is possible that the rate of $CH_4$ production was limited by the availability of methanogenic substrates rather than by the number of methanogenic bacteria (Mayer & Conrad 1990). Hydrogen in particular, may be a limiting substrate whose supply is increasing with temperature because of the different temperature characteristics of fermenting and methanogenic bacteria in anoxic paddy soil (Conrad et al. 1987). This resulted in higher $E_a$ values, when the temperature response of $CH_4$ production was measured in the presence of saturating $H_2$ which decoupled the activity of the methanogens from that of the fermenters (Conrad et al. 1987). However, the values were still well below those in the field.

*Microbial community changes*

The summing up of the temperature characteristics of different populations may result in an overall apparent activation energy which is different from that of the individual populations, especially when the population exhibit different temperature optima. Different temperature optima were reported for $H_2$-utilizing (28 °C) and acetate-utilizing (20 °C) methanogens in peat (Svensson 1984), and also for $H_2$-utilizing methanogens (35 °C) and $H_2$-utilizing homoacetogens (18 °C) in anoxic paddy soil and lake sediment (Conrad et al. 1989a). Hence, it is possible that a relative increase of mesophilic versus psychrophilic populations would result in increased $E_a$-values. However, $E_a$ for the overall community cannot become higher than $E_a$ for the individual populations and thus cannot explain $E_a$ values of up to 440 kJ mol$^{-1}$ $CH_4$ under field conditions.

*Oxidation of methane*

The temperature characteristics of the $CH_4$ emission is not only influenced by the $CH_4$-producing but also by the $CH_4$-oxidizing microbial communities (Holzapfel-Pschorn et al. 1985; Schütz et al. 1989b). Although the temperature characteristics of the $CH_4$-oxidizing community are unknown, the effect of $E_a$ of $CH_4$ emission may be substantial as $>90\%$ of the produced $CH_4$ may be oxidized before it reaches the atmosphere (Schütz et al. 1989a). The potential effect of $CH_4$ oxidation so far can only be assessed indirectly by comparing the $E_a$-values measured in laboratory experiments with rice cultures (with $CH_4$ oxidation) and with anoxic paddy soil (without $CH_4$ oxidation). Two sets of experiments with rice cultures incubated at different temperatures gave $E_a$-values of 41 and 53 kJ mol$^{-1}$ for $CH_4$ emission. These values are at the lower end of values obtained for $CH_4$ production measured in the laboratory as well as for $CH_4$ emission measured in the field.

*Diel flux changes due to environmental variables other than temperature*

Therefore, we do not believe that direct or indirect temperature effects on $CH_4$-producing and $CH_4$-oxidizing microbial populations can fully explain the high $E_a$ values in July and August. Although the calculations of these $E_a$ values were based on significant flux-temperature correlations, they probably were largely caused by diel changes of soil conditions other than temperature itself. We speculate that two different effectors may cause diel changes in $CH_4$ emission: $O_2$ availability, and root exudations.

*$O_2$ Availability*

$O_2$ availability in the rhizosphere may exhibit diel rhythms which are triggered either by light intensity or soil temperature. Diel rhythms of $O_2$ availability in the rhizosphere could be caused by changes in the transport of $O_2$ to the roots, in the $O_2$ consumption by root respiration, and in the $O_2$ consumption by aerobic microorganisms in the rhizosphere (Sculthorpe 1967; Oremland & Taylor 1977; Sand-Jensen et al. 1982). Less $O_2$ in the rhizosphere could result

in a decreased efficiency of $CH_4$ oxidation and an overproportional emission of $CH_4$ (Conrad 1989). If the availability of $O_2$ would decrease in parallel to the diel increase of soil temperature, this would result in high $E_a$ values. However, it is presently unknown whether there are diel fluctuations of $O_2$ in the rhizosphere of rice paddy fields and whether the $O_2$ concentrations would decrease with increasing temperature.

*Root exudation*

A diel rhythm of exudate supply also could stimulate a diel rhythm of $CH_4$ production in the substrate-limited methanogenic community and result in increased $E_a$ values. This conclusion is consistent with the observation that high $E_a$- values were not related to the seasonal change of the average daily soil temperatures, but seemed rather to be related to the progress of rice growth. The idea that substrate concentration influences the $E_a$ value of $CH_4$ production is also consistent with a recent observation by Westerman et al. (1989). These authors showed that the apparent activation energy of $H_2$ or acetate utilization by *M. barkeri* increases with substrate concentration. This increase was caused by a similar temperature dependence of both $V_{max}$ and $K_m$ of $H_2$ and acetate consumption kinetics. Therefore, $E_a$ was small at low *in-situ* concentrations of $H_2$ or acetate, but increased by a factor of about three at saturating concentrations of $H_2$ or acetate.

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