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SOIL CONTROLLING FACTORS OF METHANE GAS PRODUCTION FROM FLOODED RICE FIELDS IN PATI DISTRICT, CENTRAL JAVA

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ABSTRACT

Atmospheric methane (CH_4) is recognized as one of the most important greenhouse gases. Methane, with some 15-30 times greater infrared-absorbing capability than CO_2 on a mass basis, may account for 20% of anticipated global warming. Soils are one of the key factors, which play an important role in CH_4 production and emission. However, data on CH_4 emission from different soil types and the characteristics affecting CH_4 production are lacking when compared to data on agronomic practices. This study was conducted to investigate the potential of CH_4 production of selected soils in Java, and determine the limiting factors of CH_4 production. The results showed that addition of 1% glucose to the soils led to an increase in CH_4 production by more than twelve fold compared to no glucose addition. The CH_4 production potential ranged between 3.21 and 112.30 mg CH_4 kg^{-1} soil. The lowest CH_4 production potential occurred in brown-grayish Grumosol, while the highest was in dark-gray Grumosol. Chemical and physical properties of the soils have great influence on CH_4 production. Stepwise multiple regression analysis of CH_4 production and soil characteristics showed that pH and the contents of Fe_2O_3, MnO_2, SO_4, and silt in the soil strongly influenced CH_4 production. Results of this study can be used for further development of a model on CH_4 emission from rice fields.

[*Keywords*: methane, rice fields, soil chemicophysical properties, Central Java]

INTRODUCTION

Methane (CH_4) is one of the important greenhouse gases in the atmosphere (Dlugokencky *et al*., 1994). Without the presence of the greenhouse gases, the air temperature of the earth's surface would be 2-3 times lower than the actual temperature we experience now. The increase of CH_4 in the atmosphere contributes to global warming and affects the chemical changes in the atmosphere (Cicerone and Oremland, 1988; GEIA, 1993; Khalil and Shearer, 1993; IPCC, 1996). Rice fields are one of the major CH_4 sources (Cicerone and Shetter, 1981; Sass *et al*., 1990; Rennenberg *et al*., 1992; Neue and Roger, 1994; Wassmann *et al*., 1995; Neue and Sass, 1998; Wassmann *et al*., 1998). The rice paddy environment, e.g., soil, water, and the rice plant, is actively implicated in CH_4 production, oxidation, and transportation (Seiler *et al*., 1984; Holzapfel-Pschorn *et al*., 1985; Schultz and Seiler, 1989; Neue *et al*., 1997).

Methane production and oxidation in flooded rice soils are regulated by various microorganisms, which are controlled by biological, chemical, and physical factors of the soil environment. The rhizosphere of rice plants will affect both production and oxidation of CH_4. During the growth of rice plants, soil environmental conditions fluctuate due to changes in floodwater level, temperature, root growth, and fertilizer. In such a dynamic system, it is important to understand the factors which control CH_4 emission to the atmosphere. Soils are one of the key factors which play an important role in CH_4 production and emission. However, data on CH_4 emission from different soil types and the characteristics affecting CH_4 production are lacking when compared to data on agronomic practices.

Since the first study of CH_4 emission from a Californian rice field by Cicerone and Shetter (1981), evidence has accumulated showing that climate, organic matter amendment, water regime, rice variety, and fertilizer influence CH_4 emission from rice fields. Research on CH_4 emission in relation to these factors has been conducted extensively in some countries, i.e., the Philippines, China, United States, Japan, India, Thailand, and the Netherlands. However, data on CH_4 fluxes from different soil types and the soil characteristics controlling the production of CH_4 are still lacking. This study is important in terms of developing a model to predict CH_4 emission from rice

fields. Understanding the controlling factors on CH_4 production would facilitate developing such a model. Therefore, this study was conducted to investigate the potential of CH_4 production of selected soils in Java and to determine the soil characteristics controlling the emission.

MATERIALS AND METHODS

Laboratory experiment to determine the potential production of CH_4 from rice field soils was conducted. Eleven types of rice soils were selected from irrigated wetland areas in Pati, Central Java. The soils were collected based on the Indonesian Center for Soil and Agroclimate Research and Development (ICSARD) Soil Maps developed by Soepraptohardjo and Suwardjo (1966). Soil samples were classified based on the FAO Soil Classification. Eleven soil types identified from Pati District are brown Regosol, red Latosol, dark-brown Alluvial, gray-yellowish Alluvial, brown Latosol, gray Hydromorph Association, dark-gray Grumosol, brown-reddish Mediteranean, dark-brown Mediteranean, dark-gray Grumosol and Lithosol Association, and brown-grayish Grumosol.

Soils were randomly collected from 0-20 cm depths soon after rice crops were harvested. The soil samples collected were used to measure the potential production of CH_4 from their original organic matter sources. The soils were also treated with a reducible carbon source, i.e., glucose ($C_6H_{12}O_6$) to enhance their CH_4 production capacity and observations made on whether the initial characteristics of the soils could affect the production of CH_4. Glucose was added to the soils to ensure that carbon was not limiting in the soils.

Incubation Technique

Twenty-gram samples (air dried) of each soil type were placed in bottles of 120-ml volume. The incubation bottles consisted of glass beaker with a rubber stopper. The syringe holes for gas collection and pH/Eh electrode were arranged in series through two small holes in the stopper. The two small holes were also used to insert nitrogen gas to the headspace. Gas samples were withdrawn every 4 days and pH and Eh were recorded. To ensure maximum CH_4 production, a reducible C-source, i.e., glucose was added to all the soils; 1% of C over the weight of the soil used for incubation. In this way, the influence of soil characteristics on CH_4 production could be better observed. This is important if we want to determine the soil characteristics that control CH_4 production because not all soils contain sufficient carbon source.

All bottles were incubated anaerobically at 25°C for approximately 52 days to allow maximum process for methanogenic bacteria to produce CH_4. Distilled water (50 ml per bottle) was added to flood the soil and the bottle was tightly stoppered, therefore, there was an empty headspace of 70 ml in the bottle in which CH_4 and other gases produced during the incubation accumulated. To avoid contamination of the headspace from ambient CH_4, the empty headspace was first saturated with a CH_4-free gas of ultra-high purity (99.99% nitrogen gas) one day before a gas sample was collected.

The experiment was conducted in four series, each consisting of three soil types with four replications, with and without glucose treatment. Therefore, in total there were 24 bottles for this study.

Assessment of CH_4 Production

To ensure the release of all CH_4 produced during sampling, a magnetic stir bar was inserted in the middle of the soil surface in each bottle before the bottles were stoppered. The bottle was stirred and flushed with N_2 for 2 minutes at a flow rate of 200 ml $minute^{-1}$. At this time, CH_4 produced in the headspace was released and collected using a 5-ml syringe. This was considered as C_0 (concentration of CH_4 at time 0). For production rate measurement, 24 hours after taking C_0, the bottles were stirred again for 2 minutes and a 5-ml gas sample was withdrawn from the headspace (the headspace gas was mixed thoroughly by pushing the syringe plunger up and down at least 10 times). This was considered as C_{24} (concentration of CH_4 after 24 hours of incubation).

The differences in concentration between C_{24} and C_0 was regarded as the CH_4 production rate per day. After sampling for C_{24} concentration, the bottle was again flushed with N_2 while stirring for 2 minutes, and then the incubation processes were continued. Gas samples were collected every 4 days until 52 days of incubation. Methane concentration was analyzed using gas chromatograph (GC) equipped with a flame ionization detector (FID) and a porapak N column of 3m 80/100 mesh. The GC conditions were: (1) carrier gas flow of N_2 30 ml $minute^{-1}$, (2) 5 bars of compressed air and hydrogen pressure, (3) temperature of injection port 80°C, and (4) column temperature 110°C. A standard of 10.1 ppm of CH_4 was regularly analyzed through the GC.

Methane production rate was determined using the following equation (Lantin *et al*., 1995):

$$E = (C_{24}-C_0) \times \frac{Vh}{20\ g} \times \frac{mW}{mV} \times \frac{273.2}{(273.2+T)}$$

E : CH_4 production (mg kg^{-1} soil day^{-1})
C_0 : CH_4 concentration in time 0 (ppm)
C_{24} : CH_4 concentration after 24 hours (ppm)
Vh : Volume of headspace in incubation bottles (ml)
mW: Molecular weight of CH_4 (g)
mV : Molecular volume of CH_4 (22.41 liter at standard temperature and pressure/stp)
T : Temperature of incubator (^oC)

Chemical and Physical Analyses of the Soils

The chemical properties analyzed were total-N, P, K, Fe_2O_3, MnO_2, total-C, Ca, Mg, Na, Mn, Cu, Zn, extractable S, total-S, and CEC, whereas the physical properties were texture and bulk density of the soil, before the incubation experiment started. The soil analyses were carried out at the ICSARD, Bogor. The soils were collected randomly from ten points in every location, and mixed thoroughly to obtain composite soil sample. Results of the soil analyses are given in Table 1. Data obtained were analyzed using stepwise multiple regression (Snedecor, 1946) to determine relationship between soil properties and CH_4 production.

RESULTS AND DISCUSSION

Methane Production Potential of Various Soil Orders

The capacity of the 11 soils to produce CH_4 from its indigenous carbon source varied, and they are grouped in low, medium, and high categories. The patterns of CH_4 production from each soil during the incubation periods are given in Fig. 1. Gray-yellowish Alluvial and gray Hydromorph Association were grouped as the highest CH_4 production capacity with the total CH_4 production of 7.75 and 37.66 mg CH_4 kg^{-1} soil, respectively. Soils categorized as brown-grayish Grumosol, red Latosol, dark-gray Grumosol and Lithosol Association, brown Latosol, and dark-brown Alluvial were grouped as medium CH_4 production capacity ranging between 0.44 and 2.54 mg CH_4 kg^{-1} soil. The dark-gray Grumosol, dark-brown Mediteranean, brown Regosol, and brown-reddish Mediteranean were grouped as low production capacity ranging between 0.19 and 0.28 mg CH_4 kg^{-1} soil within the 52-day period.

Grouping the soils according to their capacity to produce CH_4 was also introduced by Wang *et al*. (1993a) and Neue *et al*. (1994). The soils were grouped based on their capacity to produce CH_4. Wang *et al*. (1993a) mentioned that the production of CH_4 is related to soil texture, reducible iron, manganese oxides, sulfates, and organic compounds. These properties affect the redox potential, which afterwards may influence the production of CH_4 by methanogenic bacteria.

Adding 1% C-glucose to the soils increased CH_4 production by at least 12 times compared with the untreated soils (Fig. 2). The dark-gray Grumosol soil produced the highest CH_4 level, while the brown-grayish Grumosol was the lowest. Methane production from the gray Hydromorph Association showed a different pattern with very high production of CH_4 without glucose addition, which dropped following the addition of glucose. This phenomenon on the gray Hydromorph Association was unclear, but it might be due to a sudden drop of pH of the soil on glucose treatment (Fig. 3). The pH drop ranged between 3.5 and 4.0, which was probably due to the accumulation of hydrogen ion from the reduction of glucose in the anaerobic condition. Methanogenic bacteria actively produce CH_4 at pH 6-7 and this drop of pH could reduce the methanogenic activity drastically. A similar result was also obtained by Morgan (1968), who showed that in a laboratory experiment, CH_4 formation dropped after 1% organic matter was added to an acidic soil. He mentioned that large amount of acetic acid and smaller amount of propionic and n-butyric acids probably resulted during incubation in anaerobic condition, which leads to the drop of soil pH.

Figure 2 shows that most of the soils analyzed produced more CH_4 on glucose treatment. The CH_4 production potentials of the soils were divided into three categories, low (3.21-10.15 mg CH_4 kg^{-1} soil), medium (22.51-61.08 mg CH_4 kg^{-1} soil), and high (86.28-112.3 mg kg^{-1} soil). These categories were based on the statistical analyses through comparing the means of the total CH_4 produced and analyzing the differences using Duncan's Multiple Range Test.

Total CH_4 production during 52 days of incubation was shown in Table 2. Without addition of glucose to the soil samples, the dark-gray Grumosol gave the lowest CH_4 production (0.19 mg CH_4 kg^{-1} soil), and after addition of glucose it produced the highest (112.3 mg CH_4 kg^{-1} soil). Before glucose was added, the CH_4 production pattern of the dark-gray Grumosol was flat. The same results were obtained on the dark-brown Mediteranean, brown Regosol, and brown-reddish Mediteranean. However, after glucose was added, the brown Regosol and dark-brown

Table 1. Physical and chemical properties of soils in Pati District, Central Java.

Location	Soil clasification (FAO)	Soil sample dried at room temperature													
		Texture (%)			Organic matter			Extrac. HCl 25%		Citrate-dithionite		Oxalate	Extrac. DTPA		
		Sand	Silt	Clay	C (%)	N %	C/N	P_2O_5 (mg 100^{-1} g^{-1})	K_2O	Fe_2O_3 (%)	MnO_2 (%)	Fe_2O_3 (%)	Mn (ppm)	Cu	Zn
Dukuhseti	Brown Regosol	52	34	14	0.57	0.07	9	329	46	4.75	0.19	1.24	289	1.5	1.2
Muktiharjo	Red Latosol	3	30	67	0.52	0.04	12	119	87	5.72	0.21	0.94	90	0.6	0.1
Pantirejo	Dark-brown Alluvial	2	38	60	2.01	0.15	13	50	33	2.09	0.02	1.09	46	3.1	1.0
Dukuh Mulyo	Gray-yellowish Alluvial	8	71	21	1.49	0.15	10	94	35	2.16	0.04	0.51	33	3.8	1.9
Jrahi	Brown Latosol	5	58	37	1.62	0.15	11	197	22	4.76	0.19	2.03	199	3.1	1.3
Plosorejo	Gray Hydromorph Association & brown-grayish Planosol	17	68	15	1.07	0.11	10	26	3	0.31	0.01	0.25	14	0.8	0.7
Ngurenrejo	Dark-gray Grumosol	14	65	21	0.71	0.06	12	330	88	2.33	0.19	0.88	273	8.4	1.5
Purwokerto	Brown-reddish Meditera nean and Lithosol	5	72	23	1.47	0.14	11	35	33	3.15	0.10	0.40	198	3.9	1.5
Wonorejo	Dark-brown Mediterranean Association	7	54	39	1.43	0.12	12	312	56	4.92	0.31	2.17	237	5.3	1.5
Banyu Urip	Dark-gray Grumosol and Lithosol Association	6	47	47	1.46	0.15	10	124	107	4.69	0.29	1.20	132	0.8	0.6
Treteg	Brown-grayish Grumosol Association	17	48	35	0.85	0.08	11	18	6	1.46	0.04	0.41	117	1.7	0.9

Table 1. Continued

Location	Soil classification (FAO)	SO_4 (ppm)		Extract. NH_4-acetate 1 N pH 7					pH		Base sat. (%)	Density (g cm^{-3})
		Total	KCl 0.25 N	Ca	Mg	K	Na	CEC	H_2O	KCl		
				(me $100g^{-1}$)								
Dukuhseti	Brown Regosol	478	54	11.18	3.87	0.10	0.50	14.39	6.61	5.41	100	1.56
Muktiharjo	Red Latosol	268	32	11.05	3.92	0.51	0.63	21.10	5.78	4.45	76	1.29
Pantirejo	Dark-brown Alluvial	1950	178	18.29	10.60	0.32	0.73	37.14	6.33	5.38	81	1.69
Dukuh Mulyo	Gray-yellowish Alluvial	1582	227	17.82	9.18	0.24	0.57	33.52	7.52	6.70	83	1.67
Jrahi	Brown Latosol	644	29	6.15	2.23	0.24	0.37	11.86	5.44	4.40	76	1.44
Plosorejo	Gray-Hydromorph Association & brown-grayish Planosol	537	32	3.03	0.69	0.06	0.13	6.82	4.53	3.66	57	1.51
Ngurenrejo	Dark-gray Grumosol	509	61	15.90	5.59	0.37	0.74	20.75	6.33	6.02	100	1.56
Purwokerto	Brown-reddish Mediterranean and Lithosol	739	32	18.83	2.49	0.31	0.24	23.04	7.15	5.99	95	1.76
Wonorejo	Dark-brown Mediterranean Association	563	68	14.45	4.34	0.17	0.39	14.32	5.59	4.67	100	1.60
Banyu Urip	Dark-gray Grumosol and Lithosol Association	1050	118	8.36	2.66	1.11	0.13	17.97	5.03	4.16	68	1.49
Treteg	Brown-grayish Grumosol Association	262	32	18.38	1.61	0.16	0.62	18.05	6.58	5.41	100	1.78

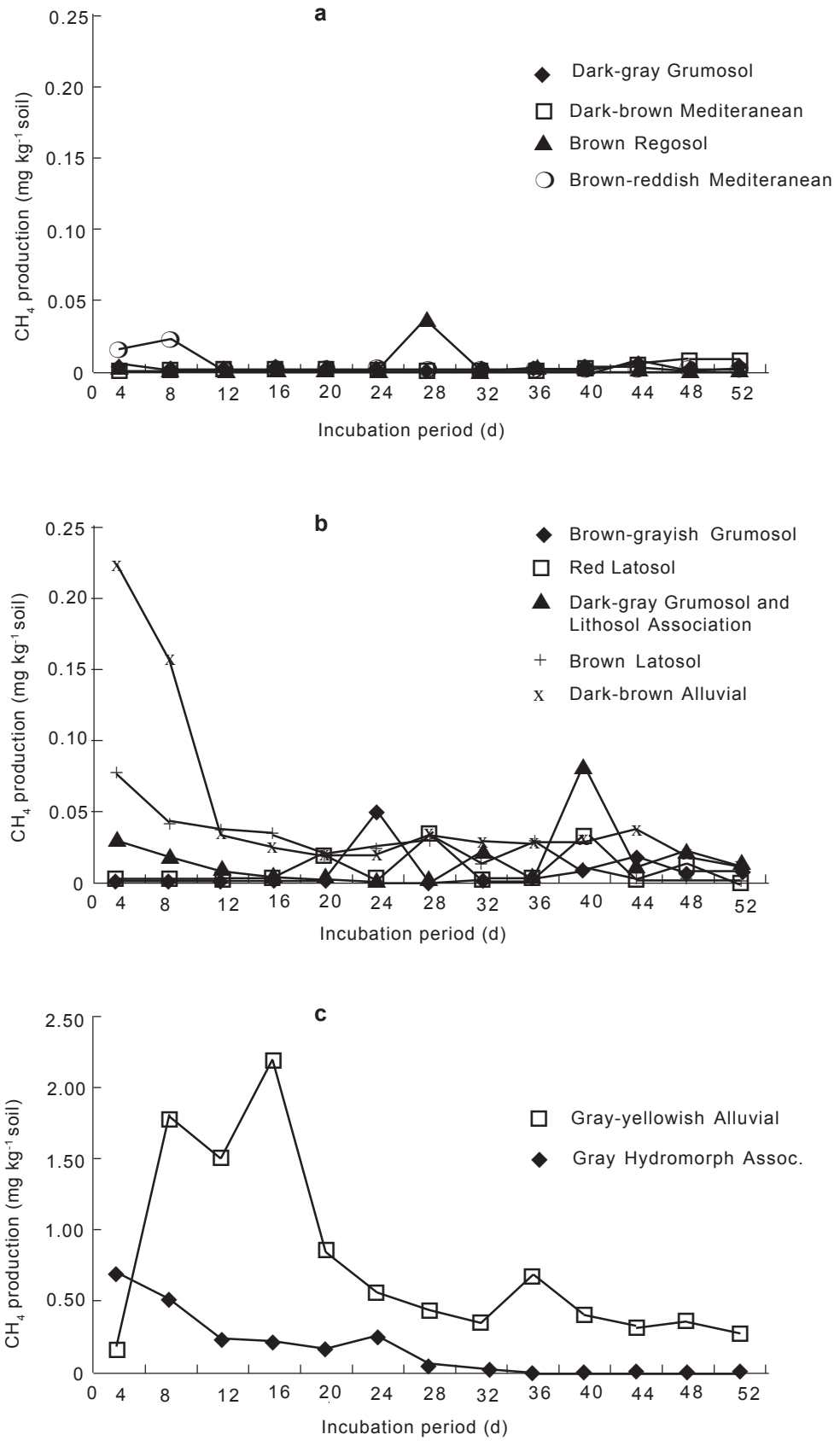

Fig. 1. Methane production pattern of the soils without addition of C-glucose during 52 days of incubation. The production pattern is divided into three groups: (a) low, (b) medium, and (c) high production of CH_4.

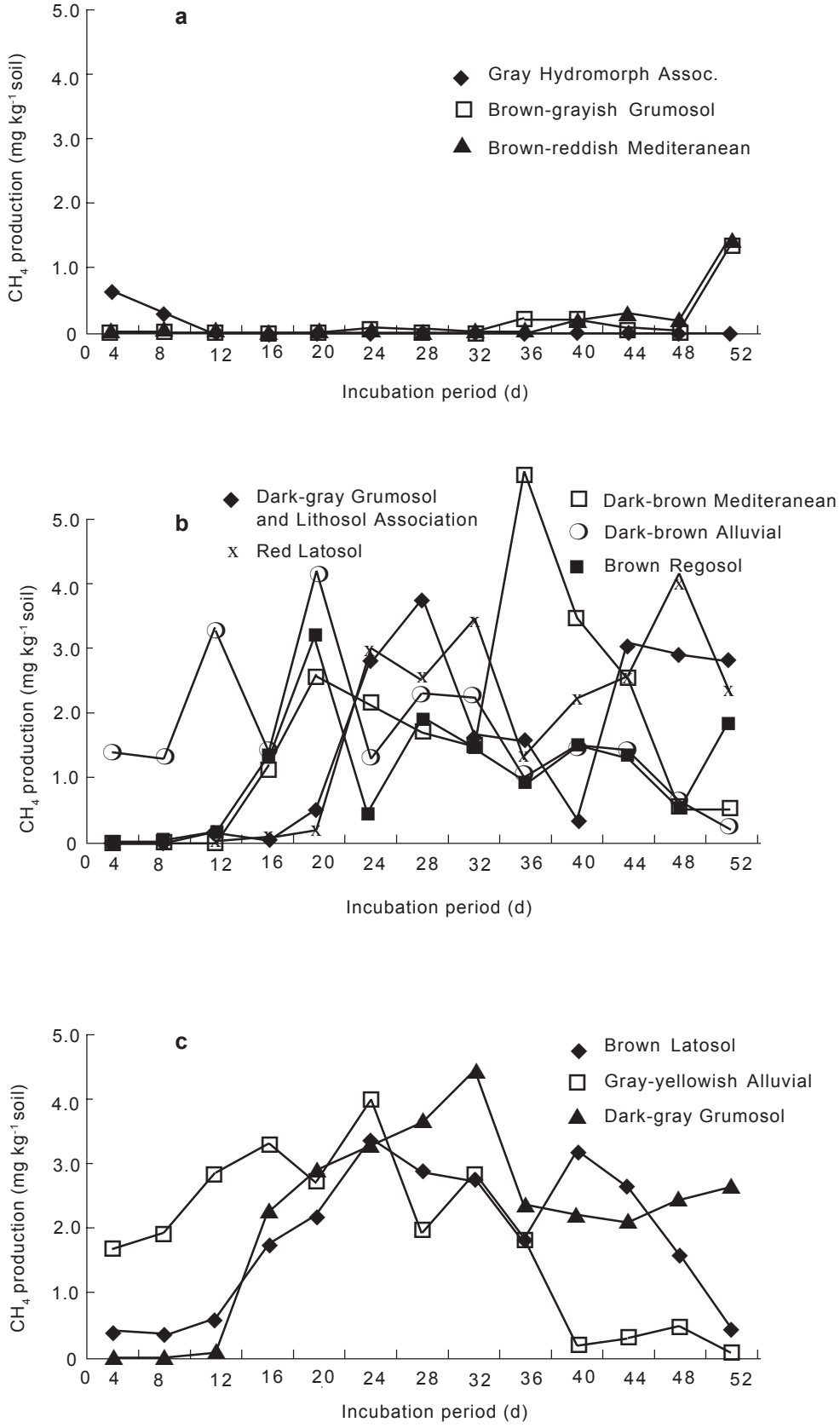

Fig. 2. Methane production pattern of the soils with addition of C-glucose during 52 days of incubation. The production pattern is divided into three groups: (a) low, (b) medium, and (c) high production of CH_4.

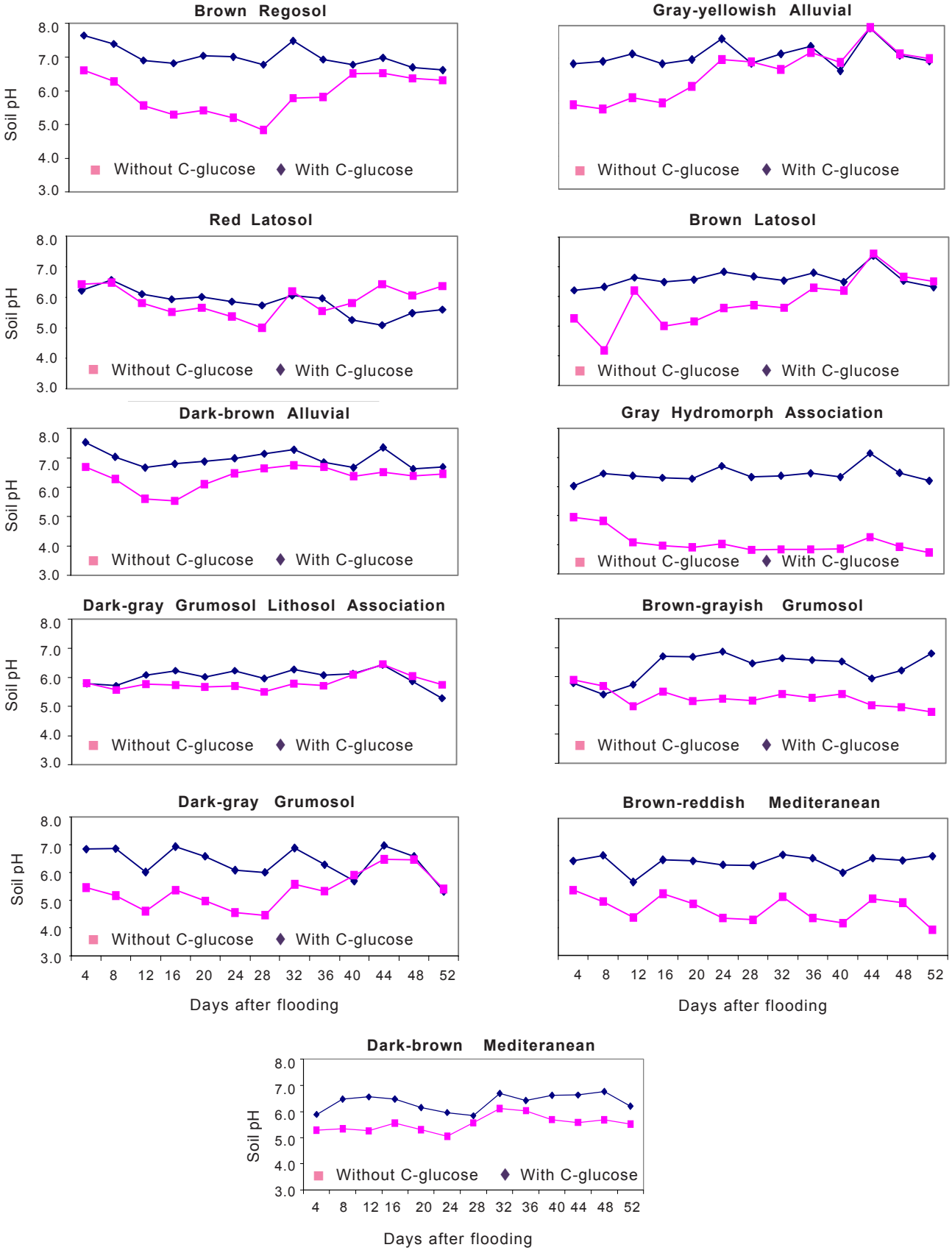

Fig. 3. pH changes of the 11 soils treated with and without C-glucose. The pH changes of the soils were recorded every 4 days for 52 days of incubation.

Table 2. Total methane production after 52 days of incubation of soils treated without and with glucose.

Soil name	Methane production (mg CH_4 kg^{-1} soil)	
	Without glucose	With glucose
Brown Regosol	0.27c	50.50c
Red Latosol	0.47c	86.28b
Dark-brown Alluvial	2.54c	86.56b
Gray-yellowish Alluvial	7.75b	94.96b
Brown Latosol	1.24c	94.71b
Gray Hydromorph Assoc.	37.66a	4.30d
Dark-gray Grumosol	0.19c	112.30a
Brown-reddish Mediteranean	0.28c	10.15d
Dark-brown Mediteranean	0.21c	87.78b
Dark-gray Grumosol and Lithosol Association	0.77c	61.08c
Brown-grayish Grumosol	0.44c	3.21d

Numbers in a same column followed by the same letter are significant at 5% level DMRT

Table 3. Characteristics of soil originating from Luzon, the Philippines.

Soil	Maahas	Luisiana	Pila
pH 1 : 1 H_2O	5.9	4.5	7.8
CEC (meq $100^{-1}g^{-1}$)	40.2	24.9	27.2
Organic C (%)	1.97	1.84	1.47
N (%)	0.166	0.18	0.182
Olsen P (ppm)	2.5	5.9	24
Active Fe (%)	1.53	4.63	0.8
Active Mn (%)	0.09	0.109	0.058
Clay (%)	66	56	21
Silt (%)	28	40	40
Sand (%)	6	4	39

Source: Denier Van der Gon *et al*. (1992)

Mediteranean exhibited an increase in CH_4 production. Methane production rate of these two soils ranged between 50 and 90 mg CH_4 kg^{-1} soil. Brown-reddish Mediteranean seems to have low CH_4 production potential even when glucose was added to the soil (10.15 mg CH_4 kg^{-1} soil). These results give an indication that soil properties influenced the production rate of CH_4 in an anaerobic soil condition.

In general the results show that addition of glucose to the soil increases CH_4 production. In practice, rice straw, which is a high carbon source, could increase CH_4 production as has been shown by Schultz and Seiler (1989). They mentioned that introducing rice straw in a reduced condition could decrease the redox potential status of the soil, and hence enhance CH_4 emission. Denier van der Gon *et al*. (1992) conducted a study at International Rice Research Institute on CH_4 emission and production from three different paddy soils of the Philippines, e.g., Pila, Luisiana and Maahas soils. The soils were treated with 1% rice straw over the weight of the soils. The soils were selected based on their different pH and some other chemical characteristics that are prone to CH_4 production. Maahas is a near neutral clay soil, Luisiana is an acidic clay soil with high iron content, and Pila is a calcareous sandy loam containing partly fragmented, mollusk shells. Results of their study showed that the CH_4 production rate in decreasing order is Pila soil > Luisiana soil > Maahas soil. The CH_4 production rate of Maahas soil was much lower than that in Pila and Luisiana, which was unexpected since Maahas soil was categorized as moderate in terms of soil characteristics for CH_4 production (active Fe, pH and organic C) (Table 3). Denier van der Gon *et al.* (1992) suggested that total organic carbon of soils did not directly correlate with CH_4 production. Therefore, other characteristics must be considered such as the chemical properties of the soils, which can influence the redox potential and pH status. In terms of soil organic carbon, determination of the soil organic fractions could possibly achieve better correlation with CH_4 production, i.e., reducible and non-reducible organic carbon.

Methane production from the brown-grayish Grumosol increased eight fold after the addition of glucose to the soil. The other soils produced higher $CH_4$of twelve to thirty fold. One possible reason is that the redox potential of the brown-grayish Grumosol soil was below the optimal condition for methanogenesis. This issue needs further study because one of the most influential redox potential buffers in this soil, i.e., the Fe_2O_3 (citrate-dithionite) concentration was also low compared to the other soils. Data in Table 1 show that the Fe_2O_3 concentration was 1.46%, which is categorized as the second lowest Fe concentration compared with the other soils. The lowest values occur in gray Hydromorph Association, i.e., 0.31%.

The addition of glucose as a source of reducible C to the soil to elucidate the controlling factors of soil characteristic on CH_4 production potential did not entirely give the expected result. The glucose concentration applied to the soils was probably too high to represent reducible carbon occurring in natural conditions (1% of the total weight of soil used for incubation), and this possibly affected the micro-environment of the flooded soils such as pH.

Application of glucose to flooded soil changed the pH of the soil. Soils with low capacity to buffer pH drop could undergo extreme change in pH to low

values, and as such, are not suitable for methanogenic bacteria. The gray Hydromorph Association exhibits this characteristic. As has been discussed previously, the extreme drop in pH value was associated with reduced CH_4 production. Although other soils reacted similarly, the pH drop was not as extreme as that shown by the gray Hydromorph Association (Fig. 3), and conditions were still tolerable for methanogenic bacteria (pH 5.0-6.0).

Determination of the Controlling Factors of CH_4 Production

Soil characteristics, such as pH, sand, silt, clay, Mg, Cu, C/N, P_2O_5, Fe_2O_3, N, SO_4, C-organic, MnO_2 were used in the stepwise multiple regression. Those parameters were involved in the reduction-oxidation processes and pH changes in soils. Using the stepwise multiple regression, five soil characteristics were found to greatly affect the CH_4 production, i.e., pH, Fe_2O_3, MnO_2, SO_4, and silt. The equation for the stepwise multiple regression is:

CH_4 production = 7.88 + 4.57 pH (H_2O) - 0.03 silt (%) - 0.015 Fe_2O_3-total (%) + 0.088 MnO_2-total (%) + 0.078 SO_4-available (ppm)

Soil pH affects the environmental conditions of methanogenic bacteria to produce CH_4. The optimum pH of paddy soils required by methanogenic bacteria is around 6.0-6.6. The same result was obtained by Wang *et al.* (1993b). The other elements, e.g., Fe_2O_3, MnO_2, and SO_4 contents in the soil affected the redox condition of soil.

Silt content of the soil highly affected the CH_4 production. Data from Table 2 show that most of the soils contained high amounts of silt, ranging from 30 to 71%. The lowest silt content occurs in red Latosol while the highest was found in gray-yellowish Alluvial. The sand distribution of the soils varied between 2 and 52%, while the clay ranged from 14 to 67%. The high content of clay occurred in dark-brown Alluvial soil while the lowest occurred in brown Regosol.

Research reported by Neue and Roger (1993) and Neue and Roger (1994) did not find the same results as obtained in Pati. They determined that reduced sandy soils with high organic carbon produced more CH_4 than clay soils with similar carbon contents. However, results from their experiment show that the active particle size distribution, i.e., clay, did not affect the production of CH_4, similar to the results obtained in Pati. The negative impact of clayey texture on CH_4 production may be caused by the formation of organo-mineral complexes. Sandy soils showed low entrapped CH_4 (Wang *et al.*, 1993b) because the pore size distribution enhances ebullition and diffusion (Neue and Roger, 1993). Methane fluxes in clayey soils may also be lower because entrapped CH_4 may be oxidized before it can escape to the atmosphere. Methane production is limited in sandy soils if water percolation and the resultant redox potential are high. Disturbances of anaerobic conditions by cultural practices, e.g., puddling, transplanting, fertilization, and weeding could release soil-entrapped CH_4 to the atmosphere. Denier van der Gon *et al*. (1992) estimated that these soil disturbances contributed to about 10% to the total CH_4 emission.

Oxidized forms of components in the soil, such as Fe^{3+}, Mn^{4+}, and SO_4^{2-}, will not be directly used as electron acceptors in biological reductions before all O_2 is released or used. After submergence, O_2 will dissolve in the flooded water and will be consumed quickly by microbes in the soil. The need for electron acceptors by facultative anaerobic and true anaerobic organisms results in the reduction of several oxidized components. Reduction of NO_3^- to NO_2^- and N_2O to N_2, Mn^{4+} to Mn^{2+}, Fe^{3+} to Fe^{2+}, SO_4^{2-} to S^{2-} and CO_2 to CH_4 will occur sequentially in flooded soil (because of thermodynamic principles) as long as available C sources exist and all entrapped O_2 is released (Patrick and Delaune, 1977). A corresponding decrease in soil Eh indicates the depletion of subsequent oxidants. For examples, nitrate is reduced to N_2O and N_2 in an Eh ranging between +250 to +350 mV. Manganic forms are reduced in slightly lower Eh range. Ferric iron reduction occurs in the range of +120 to +180 mV (Connel and Patric, 1969; Jakobsen *et al*., 1981)

Other compounds considered as micronutrients, i.e., Cu, Zn, and Mg, are probably involved in the metabolic activity of methanogenic bacteria. Their concentration in soil could enhance CH_4 production. The only reference available on the effect of micronutrients on CH_4 production was by Banik *et al.* (1995), which indicates that Zn is sensitive to methanogens at the concentration of 1-10 mg ml^{-1}. Cobalt is a constituent of cyanocobalamin, which is used for CH_4 production. Nickel is a constituent of urease, co-enzyme F_{430}, F_{420}-reducing hydrogenase, and methyl reductase. Molybdenum, a constituent of nitrogenase and NO_3^- reductase, also stimulates CH_4 production in pure cultures of methanogens and in an anaerobic digester. In a supra-optimal concentrations, these elements could possibly decrease CH_4 emission, which is presumably due to saturation of the relevant enzyme surfaces, competition for

electrons between methanogens and SO_4^{-2} and NO_3^- reducers, and the development of toxicity (Banik *et al*., 1995).

CONCLUSIONS

Addition of 1% glucose to soil samples led to an increase in CH_4 production by more than twelve fold. The CH_4 production potential ranged between 3.21 and 112.30 mg CH_4 kg^{-1} soil. The lowest CH_4 production potential occurred in brown-grayish Grumosol while the highest occurred in dark-gray Grumosol. Methane production potential of the soils without glucose addition ranged between 0.21 and 7.75 mg CH_4 kg^{-1} soil. The lowest CH_4 production potential occurred in dark-gray Grumosol while the highest CH_4 production potential occurred in gray-yellowish Alluvial. Gray Hydromorph Association does not fit in this range because of its very high CH_4 production potential (37.66 mg CH_4 kg^{-1} soil) compared with the other soils.

Chemical and physical properties of the soils have a great influence on CH_4 production. Stepwise multiple regression analyses of CH_4 production potential and soil characteristics show that soil pH and the contents of Fe_2O_3, MnO_2, SO_4, and silt in soil strongly influenced CH_4 production.

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