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BEHAVIOR OF ANAEROBIC DECOMPOSITION PRODUCTS IN SUBMERGED SOILS

Effects of Organic Material Amendment, Soil Properties, and Temperature

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The effects of soil properties, organic materials applied as amendment, and temperature on the behavior of anaerobic decomposition products of organic matter in submerged soils were studied. Methane and carbon dioxide as final products; acetic, butyric, propionic, isovaleric, *p*-hydroxybenzoic, vanillic, *p*-coumaric, ferulic, and sinapic acids, and methanol, ethanol, acetaldehyde as intermediate products were detected during the incubation, and the time course of their formation and decomposition was investigated. Soil amendment with either rice straw or green manure enhanced the formation of carbon dioxide, methane, and acetic acid. Green manure application enhanced the formation of isovaleric acid and alcohols, whereas rice straw application enhanced the formation of phenolic acids. Compost amendment did not enhance the formation of anaerobic decomposition products. Incubation at 20°C inhibited methane formation remarkably, resulting in an increase in the accumulation of volatile fatty acids and alcohols. Incubation at 35°C promoted the formation of carbon dioxide and methane remarkably, accelerated the formation and decomposition of intermediate products, and increased the amount of accumulated acetic acid in non-amended soils and that of acetaldehyde in amended soils. The pH and the content of active iron oxides and easily reducible manganese seemed to affect greatly the time course of anaerobic decomposition of organic matter in soils.

Key Words: anaerobic decomposition, organic matter amendment, volatile fatty acids, phenolic acids.

Organic materials such as algae, azolla, straw, and green manure are important nutrient sources for rice in the tropics and in China. In intensive rice cultivation in the temperate zone, organic materials have been used to maintain soil fertility and high rice yields. However, the application of organic matter sometimes exerts adverse effects on the rice plant under specific conditions. An variety of toxic substances produced under anaerobic conditions has been studied in connection with the growth of the rice plant (*22*). The rate, course, and products of the decomposition of organic

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matter affect the occurrence of these hazards. The kind of organic matter added, the soil characteristics, and temperature affect the pattern of anaerobic decomposition (*15*). Since ACHARYA (*1–4*) studied the anaerobiosis of rice straw, many studies on the subject have been conducted, as reviewed by WATANABE (*25*). To implement the IRRI policy for the recommendation of organic matter application in rice farming systems (*12*), survey and evaluation of the effect of these toxic products were essential. This study was undertaken to clarify the effects of organic matter amendments, soil properties and incubation temperature on the behavior of anaerobic decomposition products of organic matter when a moderate level (5 t/ha) of compost, rice straw, and green manure was applied to three paddy soils differing in pH, Fe content and in other characteristics.

MATERIALS AND METHODS

1. Soils. Three paddy soil samples were collected from the experiment field of the International Rice Research Institute at Los Baños (Maahas), Laguna, Philippines, and from farmer's fields in the surrounding towns of Pila and Luisiana. The physical and chemical properties of the soils are listed in Table 1. Of the three soils, the Pila soil had the highest pH, CEC, level of exchangeable calcium, and total nitrogen, while in the Luisiana soil these parameters exhibited the lowest values. Maahas clay showed intermediate values. Contents of active iron (*6*) and clay were highest in the Luisiana soil and lowest in the Pila soil. Sand content was highest in the Pila soil and lowest in the Luisiana soil. Total carbon content was higher in the Pila soil (2.48%) than in the Maahas (1.43%) and Luisiana (1.56%) soils. None of the soils contained free carbonates. Levels of exchangeable magnesium, exchangeable manganese, and easily reducible manganese were considerably higher in the Maahas soil. Montmorillonite was dominant in the clay fraction of the Pila soil. Amorphous minerals were dominant in the clay fractions of the Maahas and Luisiana soils. Allophane does not seem to exist in these soils because the pH(NaF) of the soils was lower than 9.4 (Table 1). (Allophane test was carried out according to the methods of FIELDS and PERROT (*8*). Soils were air-dried and passed through a 2 mm sieve.

2. Organic materials. Rice straw, rice straw compost, and green manure (*Gliricidia sepium*) were mixed with the soil. Compost was prepared by mixing 26 kg rice straw, 330 g $(NH_4)_2SO_4$, 600 g calcium superphosphate, and 370 g lime. The mixture was piled on the field and watered every 3 days for 2 months. The leaves and stems of a legume plant, *Gliricidia sepium*, were used as green manure. The chemical constituents of these samples were analyzed by the method of HARPER and LYNCH (*9*) (Table 2). Organic materials were air-dried, ground, and passed through a 2 mm sieve.

3. Incubation method. Ten grams of air dried soil sample with or without 25–100 mg of organic material (compost, rice straw, or green manure) were weighed in a 50 ml Erlenmyer flask, and 20 ml water (or 40 ml in the case of volatile fatty acid analysis) was added. The level of organic matter amendment (25 mg/10 g) corre-

Table 1. Characteristics of the soil samples.

Soil property	Pila soil	Maahas soil	Luisiana soil
pH (H_2O) (10 : 25)	7.28	6.43	5.40
pH (1 N KCl) (10 : 25)	6.18	5.30	4.11
pH (1 N NaF) (1 : 50)	8.83	8.87	8.50
Total C (%)[a]	2.48	1.43	1.56
Total N (%)[b]	0.204	0.140	0.117
CEC (meq/100 g)[c]	37.7	37.3	22.8
Exchangeable Ca (meq/100 g)[c]	23.0	18.0	6.74
Exchangeable Mg (meq/100 g)[c]	8.72	10.5	5.02
Exchangeable Mn (mmol/100 g)[c]	0.16	0.65	0.49
Easily reducible Mn (mmol/100 g)[d]	1.40	3.82	1.53
Free iron oxide (active iron) (mmol/100 g)[e]	11.4	39.4	59.3
Clay (%)	37.7	49.0	60.7
Silt (%)	33.5	29.1	31.2
Fine sand (%)	25.3	16.4	7.2
Coarse sand (%)	3.5	5.5	1.0
Texture	Light clay	Heavy clay	Heavy clay
Clay mineralogy[f]	Montmorillonite	Amorphous	Amorphous

[a] WALKLEY and BLACK (*24*). [b] Determined by the Kjeldahl method. [c] PEECH *et al.* (*14*). [d] Reduced by 0.2% hydroquinone in 1 N ammonium acetate (pH 7); SHERMAN *et al.* (*17*). [e] ASAMI and KUMADA (*6*). [f] WADA (*23*).

Table 2. Chemical components of organic materials analyzed by the HARPER and LYNCH method (*9*).

Chemical component	Rice straw	Compost	Green manure
	(% of oven dry weight)		
Hot-water-soluble	7.8	11.7	21.3
Lignin	14.8	15.0	25.7
Hemicelluloses	36.8	42.5	29.8
Cellulose	37.4	5.0	22.3
Ash[a]	3.2	25.8	0.9
Protein (N% × 6.25)	3.5	10.6	20.0
Carbon	39.6	14.2	45.8
Total Ash	21.6	73.2	10.5

[a] Ash remaining after sequential extractions.

sponded to the rate of 5 t/ha. The flask was stoppered with a silicon double septum, and the inner atmosphere of the flask was replaced with argon by repeatedly deaerating the flask and flushing it with argon. The flasks were further sealed by dipping

their caps into melted paraffin. The average volume of the head space was 32.8 ml with a standard deviation of 1.34 ml. In the volatile fatty acid analysis, replacement of the air phase was omitted for convenience and the water volume was increased to 40 ml in order to minimize the effect of air. The flasks were incubated at 20 and 35°C for scheduled periods. A temperature of 35°C is representative of tropical conditions where maximum decomposition of organic matter occurs. A temperature of 20°C represents the cool-temperate conditions where the rate of organic matter decomposition is slow. In the phenolic acid analysis and the Eh measurement, the quantity of sample was increased 2.5 times: 25 g of soil and 62.5 mg of organic material were submerged in 125 ml flasks. All the experiments were carried out in duplicate.

4. Gas analyses. After incubation, the flasks were shaken for 1 min, and the head space gas (0.1 ml or 0.5 ml) was drawn out through a silicon rubber cap with a gas tight syringe previously washed with helium. The collected head space gas was then injected to a gas chromatograph. A stainless steel column (180 cm long, 6.35 mm outer diameter, 4.23 mm inner diameter) packed with Molecular Sieve 5A (30/60 mesh) was used to separate argon, nitrogen, and methane. Temperatures of the injector, column oven, and detector were 23, 23, and 110°C, respectively. A stainless steel column of the same size packed with Porapak Q (50/80 mesh) was used for the separation of carbon dioxide and methane and other hydrocarbons. Temperatures of the injector, column oven, and detector were 100, 100, and 130°C, respectively. Carrier gas was helium at a flow rate of 20 ml/min. A flame ionization detector and thermal conductivity detector were used. Varian Aerograph 1868 was used throughout the study.

5. Measurements of pH and Eh. After the analysis of the head space gas, the flask was shaken again vigorously by hand for 1 min, and the silicon septeum was removed. A combined glass-calomel electrode was immersed in the soil suspension, and the pH value was read with a pH-meter after 3 min. For the measurement of the oxidation-reduction potential, a bright platinum electrode fixed with a rubber stopper was inserted into the soil layer and incubated for the whole incubation period without being taken out from the flask. The potential measured against a saturated calomel electrode using a KCl-agar bridge was transformed into that against a hydrogen electrode.

6. Volatile fatty acid analysis. After incubation, the supernatant in the flask was filtered through a Whatman No. 42 filter paper, and the residue was washed twice with 25 ml of distilled water and centrifuged at 7,000 rpm for 15 min. The supernatant was then filtered through a No. 42 filter paper and combined with the first filtrate. The combined filtrate was passed through a cation exchange column [9 mm inner diameter filled with IR 120 (20–50 mesh, H^+ form) to a height of 7 cm]. The eluate of the cation exchange column was collected in a 250 ml conical flask. The pH of the eluate was adjusted to 9–10 by 1–3 ml of 0.1 N NaOH and concentrated by boiling gently on a hot plate. When the eluate was concentrated to 10–15 ml, it was

transferred to a 50 ml beaker, concentrated again to 1–2 ml, and dried over silica gel in a vacuum desiccator. The dried concentrate was dissolved in 0.5 ml of 99% formic acid immediately before gas-liquid chromatography, and 2 μl were injected. A glass column (180 cm long, 6 mm outer diameter, 2 mm inner diameter) packed with 5% SP 1200 on Uniport S (80/100 mesh: purchased from Gasukuro Kogyo, Japan) was used for the volatile fatty acid analysis. The temperatures of the injector, column, and detector were 150, 100, and 180°C, respectively. Carrier gas was nitrogen at a flow rate of 20 ml/min. A flame ionization detector was used.

7. Phenolic acid analysis. After incubation, the content of the incubation flask (25 g soil + 50 ml water) was transferred into a 250 ml conical flask with 100 ml of 0.75 N NaOH. The flask was then stoppered tightly and shaken for 60 min. After shaking, 7.5 g Na_2SO_4 anhydride was added to the soil extractant mixture, the mixture was shaken for another 15 min and centrifuged at 7,000 rpm, and the supernatant was filtered through a dry Whatman No. 42 filter paper. One hundred ml of the filtrate was placed in a 250 ml conical flask and acidified by 3.2 ml of conc.H_2SO_4. Fifty ml of methylene chloride (CH_2Cl_2) and 25 μl of 0.2% *p*-chlorobenzoic acid solution in methanol (as an internal standard) were added to the acidified extract and stirred vigorously for 1 h with a magnetic stirrer with the flask stoppered tightly. The solution was left for some minutes until the upper aqueous layer and the lower emulsified layer had separated. The upper layer was decanted off and the emulsified layer was transferred to a Teflon centrifuge tube and centrifuged at 7,000 rpm for 5 min. A 40 ml portion of clear CH_2Cl_2 was carefully pipetted into a 50 ml glass beaker, and left in a fume hood overnight to allow CH_2Cl_2 to evaporate. The dried residue was transferred into a 3 ml reaction vial using a small volume of CH_2Cl_2 as solvent, and the solution was dried again under a stream of nitrogen gas. Fifty μl of silylation reagent, *N, O*-bistrimethylsilylacetamide, was added to the dried residue and the vial was stoppered tightly with a teflon coated silicon rubber septum and heated on a hot plate at 100°C for 30 min. After cooling, 2 μl of the reaction solution was injected to the gas chromatograph. A glass column (180 cm long, 6 mm outer diameter, 2 mm inner diameter) packed with 2% Silicon OV-17 on Uniport HP (60/80 mesh, purchased from Gasukuro Kogyo, Japan) was used to separate phenolic acid. Nitrogen was used as the carrier gas at a flow rate of 50 ml/min. A flame ionization detector was used. The column oven temperature was programmed to increase from 100 to 200°C at 10°C/min for the first 10 min, then to 250°C at 2.5°C/min for the following 20 min, and to be maintained at 250°C after 30 min. The temperatures of the injector and detector were both 300°C.

8. Alcohol analysis. After incubation, the content of the flask (10 g soil + 20 ml water) was centrifuged at 7,000 rpm for 15 min. The supernatant was filtered through a Whatman No. 42 filter paper and 2 μl of the filtrate was injected to the gas chromatograph. The remaining portion of the filtrate was preserved for aldehyde analysis. A glass column (180 cm long, 6 mm outer diameter, 2 mm inner diameter) packed with Porapak QS (80/100 mesh) was used. Carrier gas was nitrogen at 50

ml/min. A flame ionization detector was used. The temperatures of the injector, column oven, and detector were 200, 100, and 260°C, respectively.

9. Aldehyde analysis. The method of SELIM (*16*) was slightly modified and used. Ten ml of the filtrate was pipetted into a 50 ml conical flask. One ml of 0.25% 2,4-dinitrophenylhydrazine in 6 N HCl and 10 ml of isooctane were added, the mixture was stirred for 30 min with a magnetic stirrer and transferred to a separatory funnel to separate the isooctane layer and the aqueous layer. The aqueous layer was extracted by another 10 ml of isooctane, and the isooctane extracts were combined. The isooctane extract was then extracted twice with 10 ml of acetonitrile. Lower acetonitrile layers were combined, to which 50 μl of 0.12% butyraldehyde-2,4-dinitrophenyl hydrazone in acetonitrile was added as an internal standard, and then evaporated to dryness at 50°C with a rotary evaporator. The dried residue was transferred to a small glass vial with 2–3 ml of CH_2Cl_2. The solvent was evaporated under a stream of nitrogen after transfer. Finally, the sample volume was increased to 1 ml by adding 1 ml of CH_2Cl_2 to the vial. Five μl of this solution was injected to the gas chromatograph. The same column which had been used for phenolic acid analysis was used. Nitrogen was used as carrier gas at 50 ml/min. The temperatures of the injector and detector were 300°C. The temperature of the column oven was programmed to be maintained at 230°C for 5 min after injection, then to increase at 8°C/min for the next 5 min, and to be maintained at 270°C for the rest of the time.

RESULTS AND DISCUSSION

1. Gas formation

Carbon dioxide and methane formation was assayed for the three soils with four organic matter amendments, including the control, at two temperatures during 2 weeks (Figs. 2 and 3). Total amount of the formed CO_2 was calculated from the partial pressure of CO_2 and the pH (Fig. 1) of the soil using the following equation:

$$\begin{aligned}\text{Total } CO_2 &= CO_2 \text{ in the liquid phase} + CO_2 \text{ in the gas phase}\\ &= PCO_2 \times (10^{\log K} + 10^{(\log K + \log K_1)}/[H^+] + 10^{(\log K + \log K_1 + \log K_2)}/[H^+]^2)\\ &\quad \times 20/1{,}000 + PCO_2 \times (32.8/22.4) \times (273/298)/1{,}000\end{aligned}$$

where K is the Henry's coefficient for CO_2 ($-\log K = 1.47$ at 25°C; adapted from STUMM and MORGAN (*18*), K_1 is the first dissociation constant of $H_2CO_3^*$ [$=H_2CO_3 + CO_2(aq)$] ($-\log K_1 = 6.352$ at 25°C, *ibid.*), K_2 is the second dissociation constant of HCO_3^- ($-\log K_2 = 10.329$ at 25°C, *ibid.*). The volume of the head spece was 32.8 ml and the volume of the liquid phase was 20 ml, respectively. Constants at 25°C were used because both the replacement and the gas-chromatographic analysis of the head-space gas were carried out at this temperature. Partial pressures of CO_2 (PCO_2) and CH_4 (PCH_4) were calculated from the peak height ratios of CO_2/Ar and CH_4/Ar. The amount of dissolved methane was also calculated from the PCH_4 using the Bunsen's

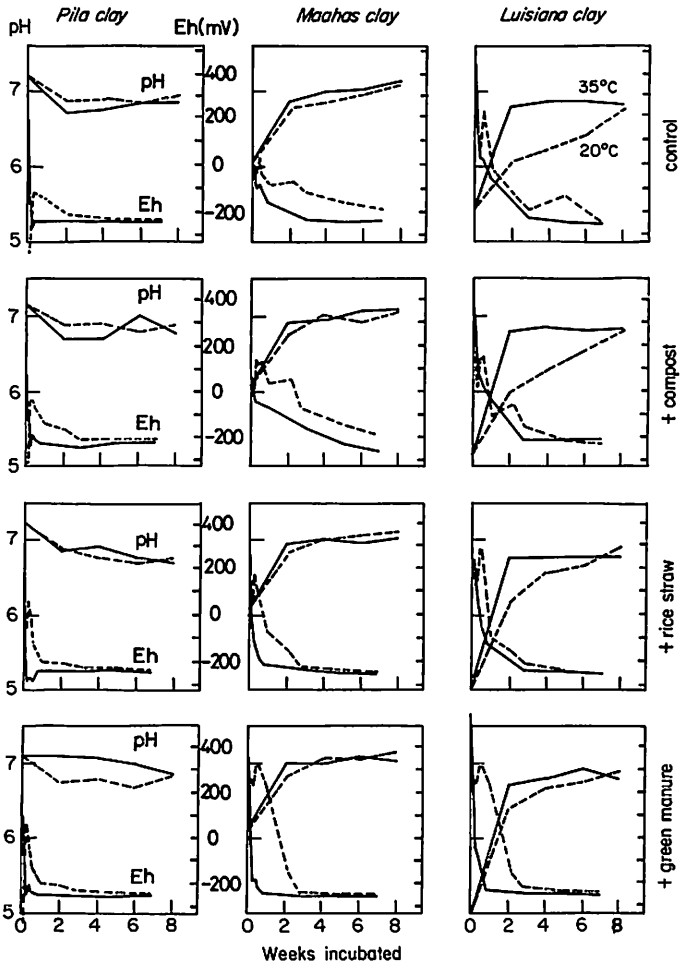

Fig. 1. Change in pH and Eh in submerged soils as affected by temperature and added organic materials.

coefficient for CH_4 (0.0301 ml/ml at 25°C) but the amount of dissolved methane obtained was nearly one-hundredth of the value of the methane in the gas phase.

For the measurement of gas formation from soils amended with fresh organic matter and incubated at 35°C, the incubation method used in this study was found to be somehow inadequate, because the inner pressure of the flask became too high and some leakage of the formed gasses occurred due to the small head space volume. However, by using the ratios of CO_2/Ar and CH_4/Ar for calculation, this error could be attenuated to some extent, because argon functioned as an internal standard. This method seemed to be suitable for the gas analysis of samples incubated at 20°C.

Carbon dioxide was the main gaseous product in all the soils and with all the

treatments at 20°C (Fig. 2). On the other hand methane formation was much lower than the carbon dioxide formation in all the soils at 20°C; methane formation was negligible in the control and compost treatments of the Maahas and Luisiana soils and an appreciable amount of methane was formed only when green manure and rice straw were added to the Pila soil (Fig. 3). Although rice straw and green manure amendments enhanced slightly the methane formation in the Maahas and Luisiana soils at 20°C, carbon dioxide still remained as the main product.

By raising the temperature to 35°C, the formation of carbon dioxide and especially that of methane were enhanced. At 35°C, a considerable amount of methane was formed even in the control and compost amended soils. The difference between the control and the compost treatment, however, was not significant in all the soils. Even if the low carbon content of the compost was taken into consideration, the compost did not seem to contribute significantly to the methane formation.

On the other hand, rice straw and green manure amendments enhanced the formation of methane remarkably, whereas only slightly the formation of carbon dioxide. As shown in the change of pH and Eh during incubation (Fig. 1), this phenomenon

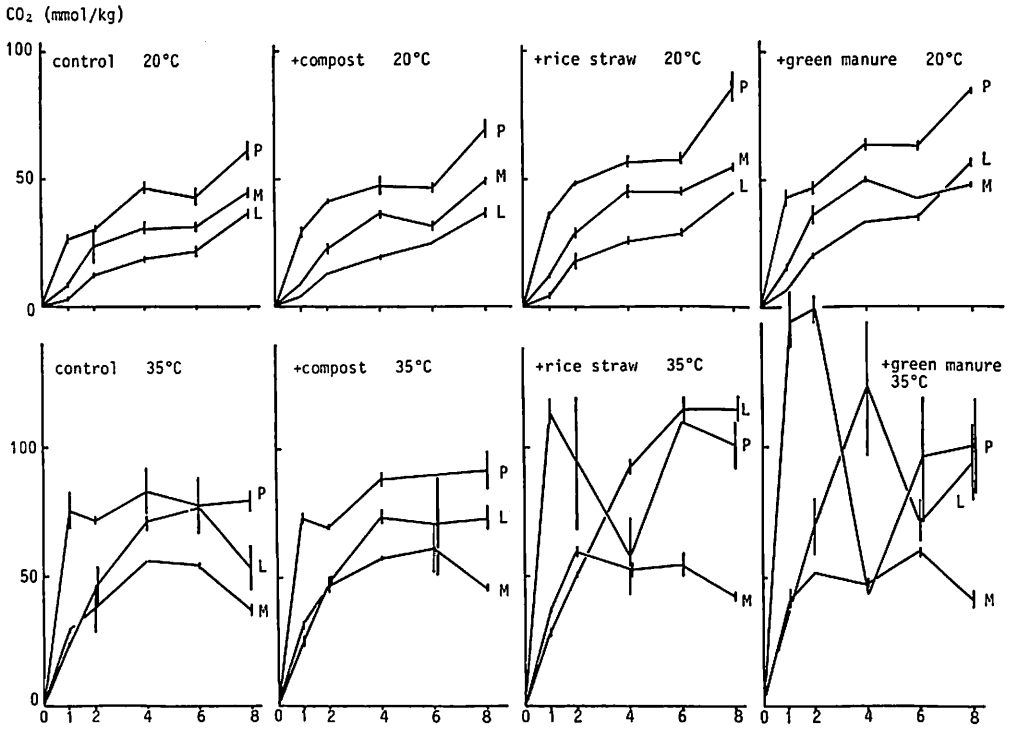

Fig. 2. CO_2 formation in submerged soils as affected by temperature and added organic materials. Bars indicate the deviation between duplicated treatments.

may be attributed to the highly reduced state when a Eh value of less than −200 mV appeared very rapidly in the amended soils at 35°C, where strict anaerobiosis including methanogenesis requires such a level of redox potential (*20*).

At 35°C, a decrease in the amount of total carbon dioxide was observed during incubation in all the soils, due to the transformation of carbon dioxide to methane (*19*). This phenomenon occurred fiirst in the Pila soil followed by the Maahas and Luisiana soils. This order was considered to correspond to the order of the establishment of the reduced state as shown in Fig. 1.

The amounts of carbon evolved as carbon dioxide and methane and the percentage decomposition of the added organic materials and native soil organic matter were calculated (Table 3). The percentage decomposition of the added organic materials varied with the soil, organic material, and incubation temperature. The values obtained, however, should be regarded as apparent and relative ones, because organic materials for amendment should also have enhanced the decomposition of native soil organic matter (*5*), and because there may have been an error due to the leak especially at 35°C.

At 20°C, the percentage decomposition of native soil organic matter ranged from 3.0 to 3.7% in the control treatments. The percentages of the decomposition of compost, rice straw, and green manure decreased in the order of the Pila, Maahas, and Luisiana soils, which corresponded to the order of decreasing pH at 20°C. Because

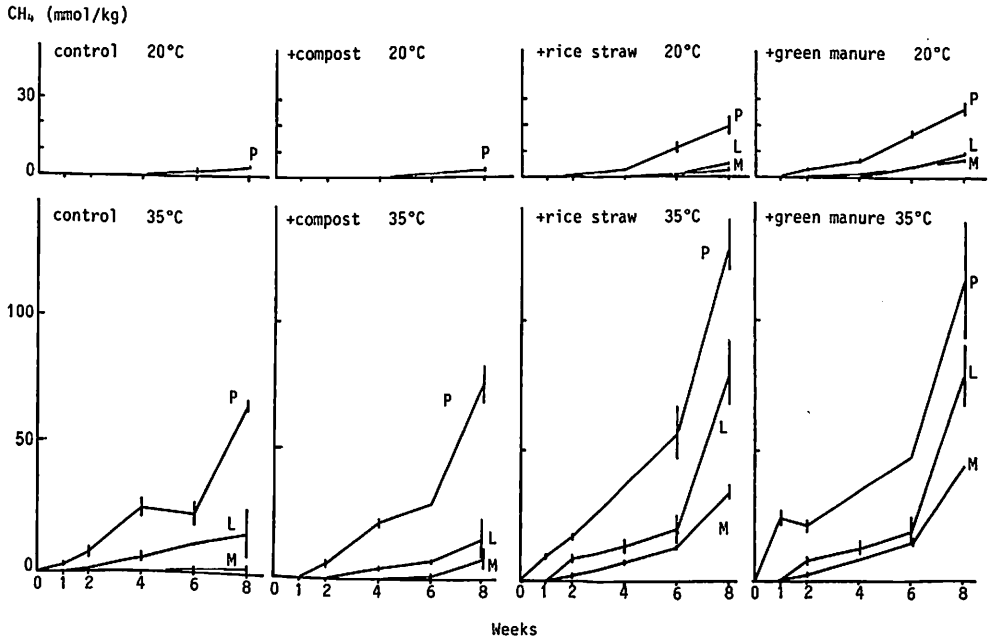

Fig. 3. CH_4 formation in submerged soils as affected by temperature and added organic materials. Bars indicate the deviation between duplicated treatments.

Table 3. Methane and carbon dioxide formed in 8 weeks of submergence and percentages of decomposition of organic materials at 20 and 30°C.

Soil treatment	Amount formed (mg/kg soil)				Increase in gasified C	Percentage decomposition (%)	
	Added C	CH_4-C	CO_2-C	(CH_4+CO_2)-C		Added organic matter	Native organic matter
At 20°C							
Pila							
Control	0	53	699	752	Control		3.01
Compost	355	31	816	847	95	27	
Rice straw	990	278	1,130	1,408	656	66	
Green manure	1,145	344	1,032	1,376	624	54	
Maahas							
Control	0	0.9	535	536	Control		3.74
Compost	355	1.2	596	597	61	17	
Rice straw	990	43	680	723	287	19	
Green manure	1,145	88	617	705	169	15	
Luisiana							
Control	0	4.3	479	483	Control		3.11
Compost	355	4.4	479	483	0	0	
Rice straw	990	59	578	637	154	16	
Green manure	1,145	120	669	789	306	27	
At 35°C							
Pila							
Control	0	762	979	1,741	Control		7.03
Compost	355	778	1,112	1,890	149	42	
Rice straw	990	1,399	1,272	2,671	930	94	
Green manure	1,145	1,540	1,388	2,938	1,197	105	
Maahas							
Control	0	41	450	491	Control		3.43
Compost	355	47	548	595	104	29	
Rice straw	990	420	510	930	439	44	
Green manure	1,145	513	537	1,050	559	49	
Luisiana							
Control	0	280	702	982	Control		6.29
Compost	355	264	887	1,151	169	48	
Rice straw	990	793	1,442	2,235	1,253	127	
Green manure	1,145	1,057	1,282	2,339	1,357	119	

the soil pH changed slowly at 20°C (Fig. 1), the initial difference in soil pH affected remarkably the organic matter decomposition. The dependence of organic matter decomposition on the soil pH has been reported by many authors (*5*, *13*).

By increasing the temperature to 35°C, the percentages of decomposition of rice straw and green manure in the Luisiana soil, which were the lowest among the three soils at 20°C, even exceeded those in the Pila soil. The increase in the methane formation in the Luisiana soil, which was ascribed to the rapid increase of soil pH and to the early establishment of a reduced state at 35°C (Fig. 1), contributed to the increase in the percentage decomposition.

At 35°C, the percentages of decomposition of added organic materials and native soil organic matter were lowest in the Maahas soil at 35°C for unknown reasons, as the Maahas soil showed intermediate values in many important soil properties (Table 1). Though the Maahas soil contained considerably large amounts of easily reducible manganese and exchangeable magnesium, it remains to be determined whether this condition would affect significantly the aanerobiosis processes.

On the other hand, the active formation of carbon dioxide and methane in the Pila soil at 20 and 35°C may be attributed to the slightly alkaline pH, lighter soil texture, and low content of active iron. Inhibition of soil microbial activity by a heavy soil texture has been reported by ALEXANDER (*5*) and was also inferred in this study in the Luisiana soil at 20°C.

2. Behavior of volatile fatty acids

Acetic acid, propionic acid, butyric acid, and isovaleric acid were detected in the water phase of submerged and incubated soil samples, with the largest concentrations in the case of acetic acid; in comparison, concentrations of propionic, butyric, and isovaleric acids were low. However, under specific conditions, isovaleric acid accumulated appreciably. The time course of the accumulation of acetic acid varied with the soil, added organic material, and incubation temperature (Fig. 4).

In the Pila soil, a large amount of acetic acid (150–250 mg/kg soil) was detected 1 or 2 weeks after submergence at either 35 or 20°C. The accumulation ceased after 2 weeks at 35°C and after 4 weeks at 20°C. In the Maahas soil, a very small quantity of acetic acid accumulated at either temperature regardless of the application of organic materials. Maximum accumulation, below 50 mg/kg soil, occurred during the first 2 weeks of submergence. In the Luisiana soil, acetic acid persisted during a longer period of time than in the other soils. At 20°C, the Luisiana soil amended with rice straw or green manure showed two peaks of acetic acid concentration: in the first and 6th week of the incubation. The difference in the time course of acetic acid accumulation among the soils may be attributed to the chemical and physical properties of the soils, which also affected the time course of methane and carbon dioxide formation. The high pH, which is favorable to bacterial activity, may have caused the early and large accumulation of acetic acid in the Pila soil. The high content of active iron may have retarded acetic acid accumulation in the Luisiana soil by delaying the establishment of the reduced state as shown in Fig. 1. Some special property of the Maahas

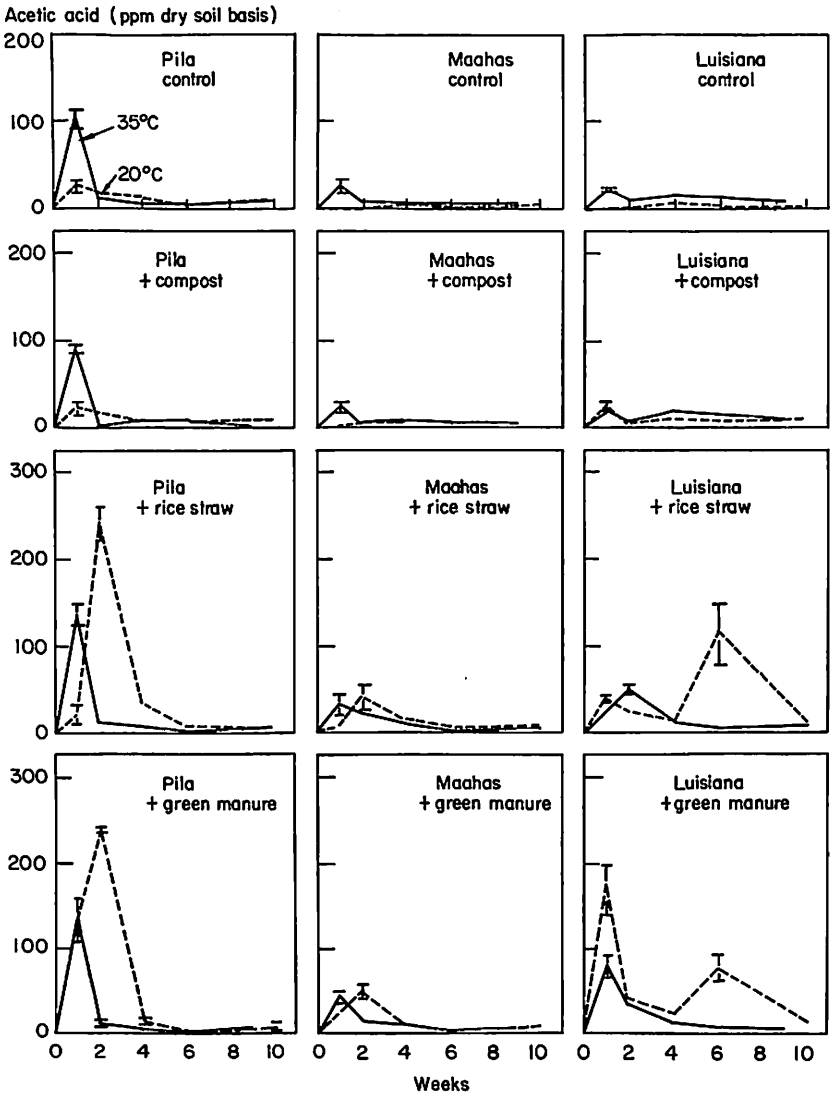

Fig. 4. Concentration of acetic acid in submerged soils as affected by temperature and added organic materials. Bars indicate the deviation between duplicated treatments.

soil, such as the high content of easily reducible manganese, which requires further examination, may have caused the low acetic acid accumulation in this soil.

Effects of the temperature on the acetic acid accumulation were also obvious: a low temperature retarded the time course but increased the accumulation in soils amended with rice straw and green manure. It is possible to consider that at 35°C acetic acid accumulation peaked within 1 week of incubation and the amount was

equivalent to or larger than at 20°C. However, the results obtained in this study are in agreement with the relationship between the volatile fatty acid formation and the incubation temperature, as shown in the previous studies as far as soils amended with fresh organic matter are concerned (*7*, *25*). Recently INUBUSHI *et al.* (*11*) reported the occurrence of a larger acetic acid accumulation at higher temperatures in a non-amended soil. When no organic matter was applied or when only compost was applied, the amount of acetic acid accumulated was larger at 35°C than at 20°C also in

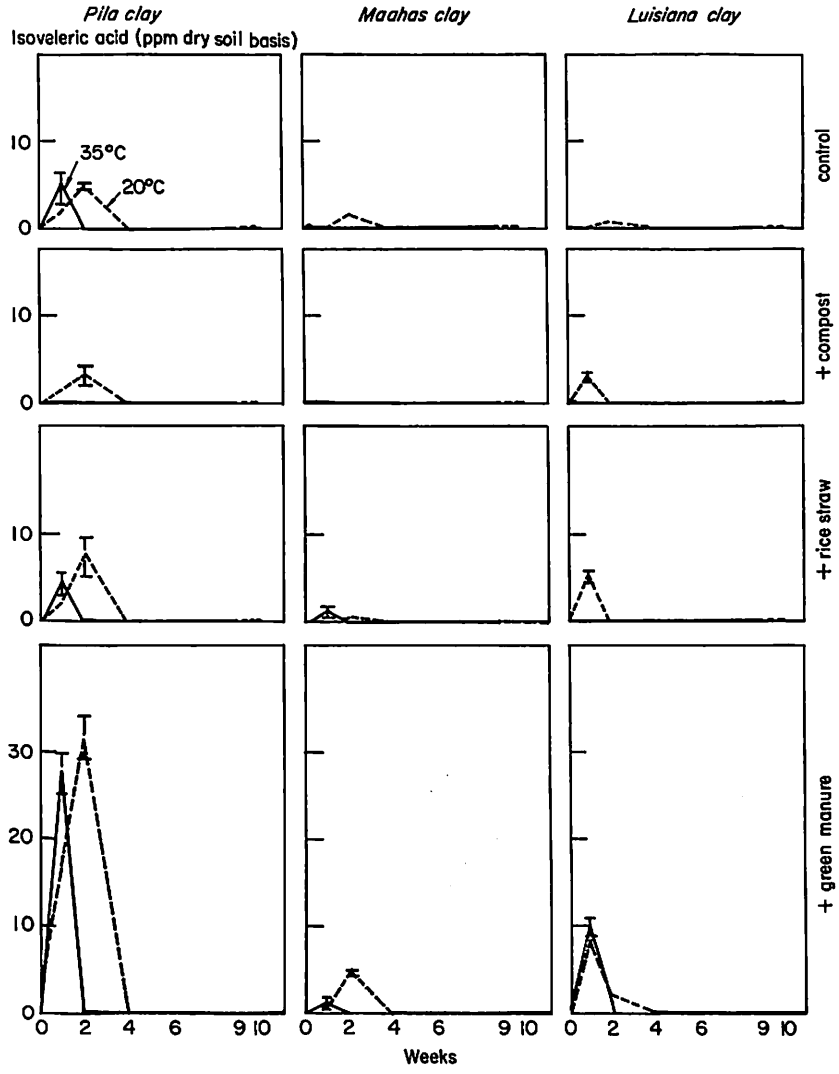

Fig. 5. Concentration of isovaleric acid in submerged soils as affected by temperature and added organic materials. Bars indicate the deviation between duplicated treatments.

this study (Fig. 4). A higher temperature may have been required to liberate the direct substrate for the acetic acid-producing bacteria from hardly decomposable soil organic matter or compost.

Large amounts of isovaleric acid accumulated when green manure was applied to the soil (Fig. 5). In soils amended with rice straw and compost, the accumulation of isovaleric acid was almost equal to or lower than that in the control. In the Pila soil, which had a high pH and light clay texture, the production of isovaleric acid was highly promoted. The temperature of 20°C was more favorable for isovaleric acid accumulation than 35°C. Slow decomposition of isovaleric acid may have enhanced the accumulation of isovaleric acid at 20°C. The peak of accumulation occurred earlier at 35°C than at 20°C. In the control and compost treatments, isovaleric acid accumulated only in the Pila soil. Isovaleric acid is a specific product of proteolytic clostridia, which obtain their energy through the Stickland reaction of branched amino acids (*21*). The observation that isovaleric acid production was enhanced only by the green manure amendment may, therefore, indicate that only green manure contained appreciable amounts of special amino acids.

3. Phenolic acid formation

The following phenolic acids were detected in the chromatogram of phenolic acids: ferulic, *p*-coumaric, *p*-hydroxybenzoic, vanillic, and sinapic acids. Levels in soils decreased in the order of Pila and Maahas soils. In the Luisiana soil, sinapic acid was the most abundant among the phenolic acids. The substance responsible for the formation of one peak, which appeared after sinapic acid, could not be identified. The amount of this compound was almost equivalent to or slightly larger than that of sinapic acid. In most cases, the level of each phenolic acid was lower than 5 mg/kg soil unless rice straw was added to the soils.

p-Coumaric, ferulic, vanillic, and sinapic acids behaved similarly (Table 4). *p*-Hydroxybenzoic acid differed partly from the other phenolic acids probably because it decomposed more easily. Rice straw amendment on the Maahas, Luisiana, and Pila soils remarkably increased the concentration of phenolic acids at 6 weeks after submergence at 20 and 35°C. However, at 2 weeks after submergence, the increase due to rice straw amendment was still limited (Table 5). In this experiment, the level of the organic amendments (0.25%) was much lower than that of native organic matter in the sample soils: Pila, 4.3%; Maahas, 2.5%; and Luisiana, 2.7%. A large part of the phenolic acids detected 2 weeks after submergence may have originated from the soil organic matter. The effect of temperature on the accumulation of phenolic acids differed among the soils. In the Pila and Maahas soils amended with rice straw, a high temperature (35°C) was more favorable for phenolic acid accumulation. In the Luisiana soil, however, a low temperature (20°C) promoted phenolic acid accumulation (Table 5). The mechanism responsible for this difference could not be accounted for. Some soil properties, such as pH, may regulate the balance of production and decomposition of phenolic acids.

Table 4. Effects of added organic materials and temperature on the concentration of phenolic acids in submerged Pila clay.

Treatment		Amount formed (mg/kg soil)						
		p-Hydroxy-benzoic	Vanillic	*p*-Coumaric	Ferulic	Sinapic	Unknown	Total
At 20°C								
Control	2 wk	1.25	0.70	2.15	4.36	0.20	1.38	10.0
	6 wk	0.23	1.03	3.86	8.02	0.15	1.01	14.3
Rice straw	2 wk	0.79	0.63	2.82	4.76	0.57	2.00	11.6
	6 wk	1.12	1.20	6.19	8.67	2.19	5.00	24.4
Green manure	2 wk	0.58	0.61	2.53	3.76	0.30	1.18	8.0
	6 wk	0.54	0.59	2.72	4.43	0.43	1.08	9.8
Compost	2 wk	0.49	0.70	2.63	4.65	0.68	1.66	10.8
	6 wk	0.57	0.74	2.96	3.99	0.64	1.29	10.2
At 35°C								
Control	2 wk	1.18	0.68	3.58	4.25	0.45	1.23	10.4
	6 wk	0.23	0.53	2.20	3.34	0.15	0.48	6.9
Rice straw	2 wk	0.94	0.57	2.31	3.45	0.48	1.42	9.2
	6 wk	0.64	1.59	6.35	8.56	2.56	5.54	25.2
Green manure	2 wk	0.65	0.67	2.38	3.72	0.55	1.46	9.4
	6 wk	0.42	0.74	3.03	4.70	0.50	1.57	11.0
Compost	2 wk	0.41	0.52	2.09	3.40	0.55	0.97	7.9
	6 wk	0.25	0.85	3.32	4.49	0.69	1.54	11.1

Table 5. Effects of added organic material and temperature on the concentration of total phenolic acids extracted from three submerged soils.

Treatment		Amount of total phenolic acids (mg/kg soil)		
		Pila soil	Maahas soil	Luisiana soil
At 20°C				
Control	2 wk	10.0	9.14	11.1
	6 wk	14.3	15.3	14.4
Rice straw	2 wk	11.6	10.0	14.4
	6 wk	24.4	23.0	36.0
Green manure	2 wk	8.0	10.6	12.8
	6 wk	9.8	8.39	11.3
Compost	2 wk	10.8	9.32	11.7
	6 wk	10.2	8.97	12.0
At 35°C				
Control	2 wk	10.4	8.73	9.82
	6 wk	6.9	9.81	8.24
Rice straw	2 wk	9.2	8.89	12.6
	6 wk	25.2	33.9	27.6
Green manure	2 wk	9.4	9.92	12.4
	6 wk	11.0	7.13	10.8
Compost	2 wk	7.9	8.98	11.4
	6 wk	11.1	10.1	14.1

Compost amendment also increased slightly the concentration of phenolic acids in all the soils at both temperatures. The increase, however, was very slight compared with the effect of rice straw amendment. This finding may indicate that the phenolic compounds in rice straw were decomposed considerably by composting.

Green manure amendment tended to depress phenolic acid formation. Concentration of these phenolic acids was lowest in the soils with green manure amendment 6 weeks after incubation, although the lignin content in green manure was higher than in rice straw (Table 2). Decomposition of phenolic acids may have been enhanced by the coexistence of easily decomposable constituents in green manure. In contrast the high production of phenolic acids from rice straw may be attributed to the fact that grasses contain large amounts of phenolic acids, which are linked to lignin through ester-bonds (*10*).

4. Formation of alcohols and aldehydes

At a 0.25% level of amendment, none of the amended organic materials produced detectable amounts of alcohols and aldehydes in the sample soils. Therefore, the behavior of the alcohols and aldehydes was studied only in the soils amended with 1%

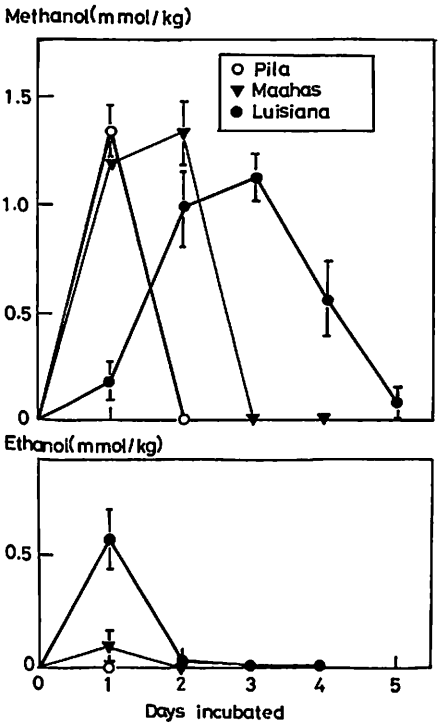

Fig. 6. Concentration of alcohols in 3 submerged soils amended with green manure (1%) at 35°C. Bars indicate the deviation between duplicated treatments.

green manure. The time course of alcohol formation in soils at 35°C is shown in Fig. 6 and at 20°C in Fig. 7. Methanol and ethanol were the only alcohols detected in the submerged soils. Methanol concentration in the Pila, Maahas, and Luisiana soils at 35°C peaked at 1, 2, and 3 days after submergence, and the peak concentrations were 1.30, 1.29, and 1.10 mmol/kg, respectively. At 20°C, methanol concentration peaked at 2, 4, and 6 days after submergence, and the peak concentrations were 1.40 mmol/kg in the Pila soil, 1.45 mmol/kg in the Maahas soil, and 1.72 mmol/kg in the Luisiana soil. Thus at lower temperatures, methanol formation was retarded but the amounts formed were slightly higher. The order in which the peak concentrations were attained corresponded to the decreasing order of soil pH and exchangeable Ca and the increasing order of active Fe and clay content.

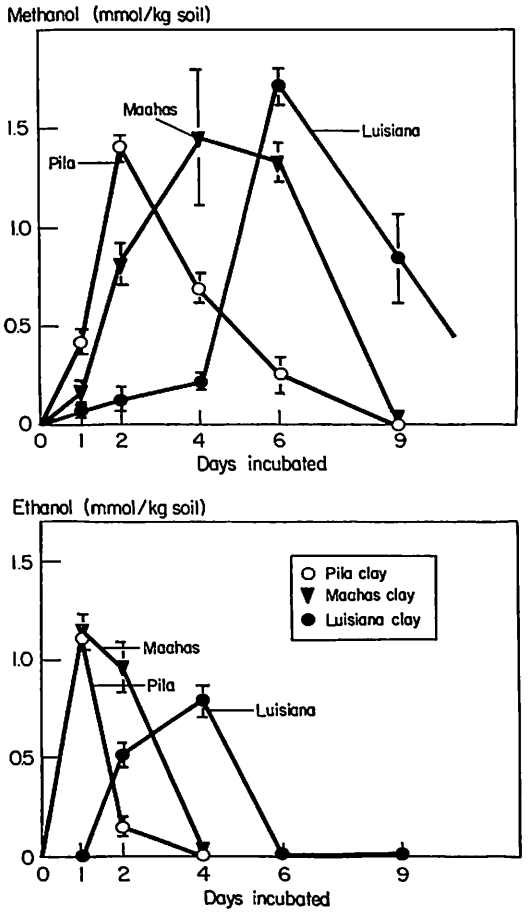

Fig. 7. Concentration of alcohols in 3 submerged soils amended with green manure (1%) at 20°C. Bars indicate the deviation between duplicated treatments.

The same trend was observed for ethanol formation. However, the appearance and disappearance of ethanol were faster than those of methanol. Ethanol accumulation at 35°C was observed only within 2 days of submergence in all the soils. The amount was largest in the Luisiana soil (0.576 mmol/kg), followed by the Maahas soil (0.087 mmol/kg) and Pila soil (0.007 mmol/kg). Thus ethanol formation in Pila clay was not appreciable at 35°C. Ethanol formation in the Pila soil may have peaked within 24 h at 35°C. Ethanol concentration at 20°C peaked after 1 day in the Pila and Maahas soils and after 4 days in the Luisiana soil. Peak concentrations were 1.10 mmol/kg in the Pila soil, 1.11 mmol/kg in the Maahas soil, and 0.79 mmol/kg in the Luisiana soil. Because ethanol and methanol accumulations were sequential, it is

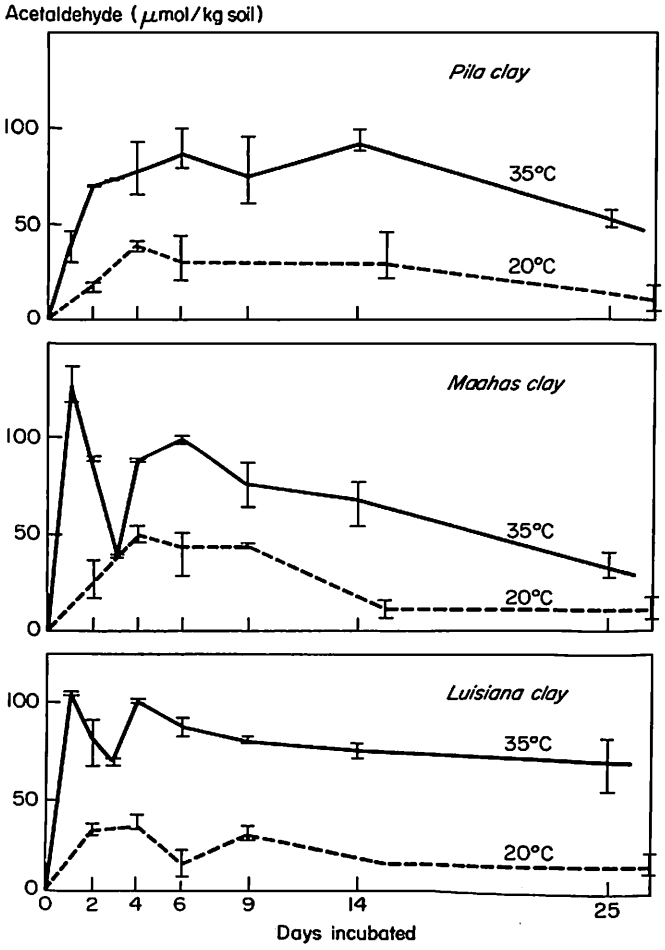

Fig. 8. Concentration of acetaldehyde in 3 submerged soils amended with green manure (1%) at 20 and 35°C. Bars indicate the deviation between duplicated treatments.

possible to consider that ethanol is a precursor of methanol in submerged soils. Judging from their very short persistence and low concentrations, the alcohols may not exert any physiological effect on rice plants grown in submerged soils.

In the chromatogram of the 2,4-dinitrophenylhydrazones of the carbonyl compounds extracted from the submerged soils, formaldehyde and acetaldehyde were identified. The peak of propionaldehyde was very small. Because butyraldehyde was not detected in the sample, it was used as an internal standard. Acetaldehyde concentration was highest among the aldehydes detected here. The amount of acetaldehyde accumulated was, at most, 0.1 mmol/kg soil but acetaldehyde persisted longer than the alcohols. The behavior of acetaldehyde in 3 submerged soils at 20 and 35°C is compared in Fig. 5. At 35°C, acetaldehyde formation showed two peaks in all the soils. After the second peak, acetaldehyde persisted until 25 days after submergence or longer. At 20°C, acetaldehyde concentration was less than half that at 35°C during incubation.

CONCLUSION

Even if a low application rate on a carbon basis is taken into account, compost amendment did not increase significantly the formation of carbon dioxide, methane, volatile fatty acids, alcohols, and aldehydes, but it slightly increased the formation of phenolic acids. Rice straw amendment, however, markedly increased the formation of carbon dioxide, methane, volatile fatty acids, and phenolic acids. The formation of phenolic acids was enhanced only with rice straw amendment. Green manure amendment increased considerably the formation of carbon dioxide, methane, volatile fatty acids (especially isovaleric acid), methyl and ethyl alcohols, and aldehydes. Green manure amendment did not enhance the formation of phenolic acids.

In soils amended with fresh organic materials, the formation of intermediate products such as volatile fatty acids and alcohols was accelerated at 35°C but the amounts formed were smaller. These intermediate products were considered to be transformed to methane quickly. On the other hand, at a lower temperature (20°C), the activity of the methanogenic bacteria was depressed, and therefore, volatile fatty acids and alcohols accumulated in larger amounts. The concentration of acetaldehyde, although small, was larger at 35°C than at 20°C. In the non-amended soil and compost-amended soil, a larger accumulation of acetic acid was observed at 35°C than at 20°C. The effect of the temperature on phenolic acid formation depended on the soil.

The rate of methanogenesis appeared to control the amount and time course of the accumulation of anaerobic decomposition products in soils amended with fresh organic materials. On the other hand, the rate of the decomposition of soil organic matter or compost to intermediate products such as volatile fatty acids and alcohols was considered to determine the kinetics of intermediate products in soils with no organic amendment or amended with compost.

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