Chapter 3

EFFECT OF SOIL PROPERTIES, ADDED ORGANIC MATERIALS AND TEMPERATURES ON THE KINETICS OF VOLATILE FATTY ACIDS AND ORGANIC REDUCING SUBSTANCES IN SUBMERGED SOILS

SUMMARY

Kinetics of acetic acid, isovaleric acid and organic reducing substance including phenolic substance were investigated in 3 submerged soils added with 3 organic materials and incubated at 20°C and 35°C. Formation of volatile fatty acid was largest in calcareous soil (pH 7.2), but it was delayed in acidic ferruginous soil (pH 5.4). Formation in Maahas clay (pH 6.0) was very little. Accumulation of volatile fatty acids was enhanced by addition of rice straw and green manure and by lower temperature, except that acetic acid formation in the control and compost treated soils were higher at 35°C. Isovaleric acid formation was prominent only in green manure treated soils, probably because it is a product of proteolytic clostridia. Formation of organic reducing substance was enhanced by added organic materials and by higher temperature. Its peak of accumulation followed that of volatile fatty acids and peaking was delayed by lower temperature. Organic reducing substance accumulated in smaller amounts but prevailed for a longer period than volatile fatty acids.

INTRODUCTION

Volatile fatty acids are major intermediate products in anaerobic decomposition of organic matter. They have been assumed to be the cause of rice injury in organic soils and peat soil (Takijima, 1963), and acidic soils especially at low temperature (Cho and Ponnamperuma, 1972). Many fundamental studies have been conducted on the kinetics of volatile fatty acids connected with gas formation and other kinetics of soil reduction (Watanabe, 1982). Phenolic acids are also potential toxic substances for rice culture and have been studied by many researchers as reviewed by Tsutsuki (1982).

Because organic matter is assumed to take an important part in the fertility management of tropical rice soils (Ponnamperuma, 1982) decomposition of organic matter should be studied from many sides. It is very important to know the amount and kinetics of toxic decomposition products in connection with applied organic materials, soil properties and temperatures. As volatile fatty acids and phenolic substances in water soluble form may be the most toxic products, their kinetics were investigated together and compared. The amount of organic reducing substances determined by Folin-Ciocalteu's reagent was assumed to stand for the approximate amount of phenolic substances in this study.

MATERIALS AND METHODS

Soils

Three wetland rice soils (Pila, Maahas, Luisiana) differing in pH, organic matter content and active iron were used (Table 1). Soils were air-dried and crushed to pass through a 2 mm sieve.

Organic Materials

Rice straw, rice straw compost, and green manure (Gliricidia sepium) were used. Compost were made by mixing 26 kg of rice straw, 330 g $(NH_4)_2SO_4$, 600 g calcium superphosphate, and 370 g lime and piling in a 1.5 m diameter circle in an open field. It was watered every

3 days and left for 2 month before application. Analysis of C and N of organic materials are given in Table 2.

Volatile fatty acids

Incubation. Ten grams of soil samples (Pila, Maahas and Luisiana) with or without 25 mg of organic materials (compost, rice straw and green manure) were weighed into a 50 ml Erlenmeyer flask, submerged by 40 ml of water and deaerated under reduced pressure. The flask was sealed with parafilm and incubated at 20°C and 35°C in incubators. Each treatment was replicated twice.

Extraction, purification and concentration. After the scheduled period of incubation the supernatant in the flask was filtered through a Whatman No. 42 filter paper, and the residue was washed two times with 25 ml of distilled water and centrifuged at 7000 rpm for 15 minutes. The obtained supernatant was then filtered through a filter paper and combined with the first filtrate.

The combined filtrate was passed through a cation exchange column (i.d. 9 mm x 7 cm) packed with IR120 20-50 mesh, H^+ form. The eluate of the cation exchange column was collected in a 250 ml conical flask. Then the eluate was neutralized to the pH value of 9-10 by 1-3 ml of 0.1 N NaOH. The neutralized eluate was concentrated by boiling on a hot plate. Enough amount of Teflon tips were added for boiling gently. When the eluate was concentrated to 10-15 ml, it was transferred to a 50 ml beaker and concentrated again to 1-2 ml. It was then dried over silica gel in a desiccator under vacuum.

Gas liquid chromatography of volatile fatty acids. The dried concentrate was dissolved in 0.5 ml of formic acid immediately before GC analysis, and 2 microliter of the concentrate-formic acid solution was injected to the gas-liquid chromatograph. A Varian aerograph model 1868 equipped with a FID detector was used. This instrument features on-column injection and direct connection of the column to the detector. A glass column (6 ft x 6 mm O. D. x 2 mm I. D.) packed with 5% SP 1200 on Uniport S (80/100 mesh) was used. Nitrogen was used as carrier gas at the flow rate of 20 ml/min. Flow rates of hydrogen and air for the FID detector were 30 ml and 300 ml/min, respectively. The temperatures of injector, column, and detector were 150°C, 100°C, and 180°C, respectively.

Organic reducing substance

The concentrate in formic acid of the water extract of incubated soil samples were dried again over silica gel under vacuum after it was analyzed for volatile fatty acids. Volume of the sample did not change practically by the organic acid analysis because the used amount was only 2 or 4 microliter.

To the dried concentrate in a 50 ml beaker, 0.5 ml of Folin-Ciocalteu's Phenol reagent (2 N) was added. After leaving it 30 minutes, 25 ml of 2% Na_2CO_3 solution was added. It was left again for more than one hour at room temperature. The developed blue color was determined at 750 nm by a Beckman uv-vis spectrophotometer.

RESULTS AND DISCUSSION

Kinetics of volatile fatty acids

Acetic acid, butyric acid and isovaleric acid were detected in water extract of submerged soils.

Acetic acid accumulation was largest in amount, followed by isovaleric acid, and butyric acid in this order. The correct amount of propionic acid could not be determined, because its peak overlapped a ghost peak.

Patterns of acetic acid accumulation differed by soils, added organic materials, and incubation temperatures (Fig. 12).

In Pila clay loam, large amount of acetic acids (150 ~ 250 mg/kg soil) accumulated after 1 and 2 weeks of submergence at 35°C and 20°C, respectively. Low amount of active iron and large amount of easily decomposable organic matter in the soil is assumed to be the cause of the large accumulation within a short period of incubation, because such conditions cause the fast development of reduced state.

In Maahas clay, acetic acid accumulated very little at both temperatures even when organic matter was added to the soil. There was a slight accumulation less than 50 mg/kg soil after 1 or 2 weeks of submergence.

In Luisiana clay, acetic acid accumulation lasted longer than in the other soils. When rice straw or green manure was added to this soil at 20°C, acetic acid accumulation showed two peaks at the first and the sixth weeks of incubation. Luisiana clay is heavy clay in texture and contains large amount of active iron. The second peak implies that acetic acid could accumulate after most of the active iron in the soil had been reduced. The acetic acid formed in the first week might have come from strictly anaerobic sites which developed locally in the soil.

Addition of compost to soils did not enhance the acetic acid formation. When soils were incubated without adding organic matter or with compost, larger amount of acetic acid accumulated at 35°C than at 20°C in all soils used here. Acetic acid accumulation showed a peak after one week of submergence at 35°C, while it showed a smaller peak or not at all at 20°C. In the control and compost treatments of Luisiana soil, acetic acid accumulation was small but lasted until the ninth week of incubation at 35°C. The larger accumulation of acetic acid in the control and compost treatments at 35°C than at 20°C may be due to the larger decomposability of indigenous soil organic matter and compost at higher temperature.

When rice straw and green manure were applied to soils, acetic acid accumulation increased remarkably especially at 20°C. In Pila clay loam and Maahas clay the peak of acetic acid accumulation was observed at the first week of submergence at 35°C, while it was at the second week at 20°C. Contrarily to the control and compost treatments, larger amount of acetic acid was accumulated at 20°C than at 35°C when rice straw and green manure were added. Of the 2 peaks which accumulated after 1 and 6 weeks at 20°C in Luisiana clay, the first peak was larger in the green manure treatment, while the second peak was larger in the rice straw treatment. In Luisiana soil at 35°C, acetic acid accumulation showed only one peak, which was at the first week in the green manure treatment while at the second week in the rice straw treatment. When rice straw and green manure were compared, green manure caused more acetic acid accumulation in a shorter period, and acetic acid accumulation lasted longer in rice straw treated soils. These results are obviously because green manure is more readily decomposable than rice straw.

Isovaleric acid accumulation was prominent when green manure was applied to soils (Figure 13). Pila soil produced significantly larger amount of isovaleric acid than the other two soils. When the temperatures were compared, 20°C was more favorable for isovaleric acid accumulation than 35°C. The peak of accumlation was observed at the first (at 35°C) and the second week (at 20°C except Luisiana soil) of submergence. In Luisiana soil, the peak of accumulation was observed at the first week at both temperatures. After 6 weeks of submergence, isovaleric acid was not detected unlike acetic acid which was formed at this period in Luisiana soil.

In the control and compost treatments, isovaleric acid did not accumulate except in Pila soil. In other soils, slight accumulation was observed only at 20°C. Behavior of isovaleric acid was different from acetic acid which accumulated in larger amounts at higher temperature in the control and compost treatments. Contribution of indigenous soil organic matter to isovaleric acid formation was very low. Isovaleric acid is a specific product of proteolytic clostridia which get energy through Stickland reaction of amino-acids (Takeda and Furusaka, 1975). It may be due to this reason that the formation of isovaleric acid was enhanced only when green manure which contains enough amount of nitrogen was added to soils.

Butyric acid accumulation was very little even if compared with isovaleric acid (Figure 14). The accumulation occurred only in the first or second week of submergence when green manure was applied to soils.

Kinetics of organic reducing substances including phenolic acids

The amount of organic reducing substances determined by Folin-Ciocalteu's reagent includes low molecular and high molecular phenolic subtances derived from the applied organic matter and from its degradation products. Because the colorimetry by Folin-Ciocalteris reagent is reactive to reducing substances, the determined value should be regarded as the total content of reducing organic substances which have as strong reducing power as phenolic substances. Interference by ferrous or manganese (II) ion was negligible, because the sample was passed through the cation exchange resin.

Phenolic acids in the water extract may have a most direct physiological effect to rice plant. However, composition of water soluble phenolic acids have not been determined well. Well defined phenolic acids such as p-coumaric acid, ferulic acid and p-hydroxybenzoic acid may occupy very small part of water soluble reducing organic substances in their free forms, or they exist combined with more hydrophilic molecules, because of their very low solubility. Their concentration were also very low even in alkaline extract of soils (Shindo and Kuwatsuka 1978).

The kinetics of organic reducing substances in 3 soils applied with 3 organic materials were compared at 20°C and 35°C (Fig. 15).

The level of organic reducing substance was largest in Pila clay loam, followed by Luisiana clay and Maahas clay in this order in every treatment. Additions of compost, rice straw, and green manure all enhanced the accumulation of reducing substance. Patterns of accumulation were similar among the control, compost and rice straw treatments, but it was different in the green manure treatment.

In control, compost, and rice straw treatments, larger amount of reducing substance accumulated at higher temperature (35°C). At 35°C, the peak of reducing substance accumulation was found after 2 weeks of incubation except the control and compost treatments of Maahas clay which had no peak of accumulation. In some cases, the peak of accumulation was also seen after 6 weeks of incubation.

At 20°C, the accumulated amount of reducing substance was lower than that at 35°C in every soil and the peak of accumulation was seen after 4-6 weeks of submergence in Pila and Maahas soils. The level was still gradually increasing after 10 weeks of submergence in the control and compost treatments of Luisiana clay at 20°C.

The peak of accumulation after 2 weeks of submergence at 35°C may be due to the anaerobic decomposition of organic matter. Higher temperature caused larger formation of decomposition products in shorter period, and lower temperature decreased and retarded the formation as seen in Figure 15.

Compared with the kinetics of volatile organic acids, formation and degradation of reducing substance in submerged soils were slower than that of volatile fatty acids. This may be because these reducing substances including phenolic acids were split from hardly decomposable fraction of organic matter in soils.and phenolic acids themselves are more stable than volatile fatty acids in soil and have a longer turn over time. Larger accumulation at 35°C may be also due to the stableness of these decomposition products.

When green manure was added to soils, the accumulated amount of reducing substance was larger at 20°C than at 35°C. At 20°C, accumulated amount of reducing substance was largest and the peaking was earliest in the green manure treatment in every soil. The level of reducing substance was highest already at the first week and it decreased gradually with time (in Pila and Maahas soils) or decreased after the plateau which lasted for 4 weeks (in Luisiana clay). This abundance of reducing substance in the green manure treated soils incubated at 20°C may be due to the readiness in decomposability of green manure and to the stableness of decomposition products at low temperature. However, at 35°C, the level of reducing substance at the peak in green manure treated soils was lower than that in rice straw treated soils (in Pila and Maahas soils) or it decreased with time after it showed the highest level at the first week (in Luisiana clay). This may be because decomposition products of green manure are more susceptible to further decomposition at 35°C than those of rice straw or compost.

Accumulated amounts of water extractable reducing substances were at most 35 mg/kg in Pila clay loam, 20 mg/kg in Maahas clay and and 30 mg/kg in Luisiana clay. They were much lower than the peak concentration of acetic acid which reached 250 mg/kg in Pila clay loam, 50 mg/kg in Maahas clay and 175 mg/kg in Luisiana clay. However, phenolic acids are toxic to plants at lower concentrations than volatile fatty acids. The lowest concentration of phenolic acids causing injury to various plants have been found to be at the order of 0.1 m M or 10 ppm level (Takijima, 1963). Therefore, if phenolic acids were dominant constituents of reducing substances, the possibility of inhibition can not be expelled. In addition, because the peak of reducing substance formation came after that of volatile fatty acid and lasted longer, it may prolong the adverse effect of organic matter decomposition in submerged soils.

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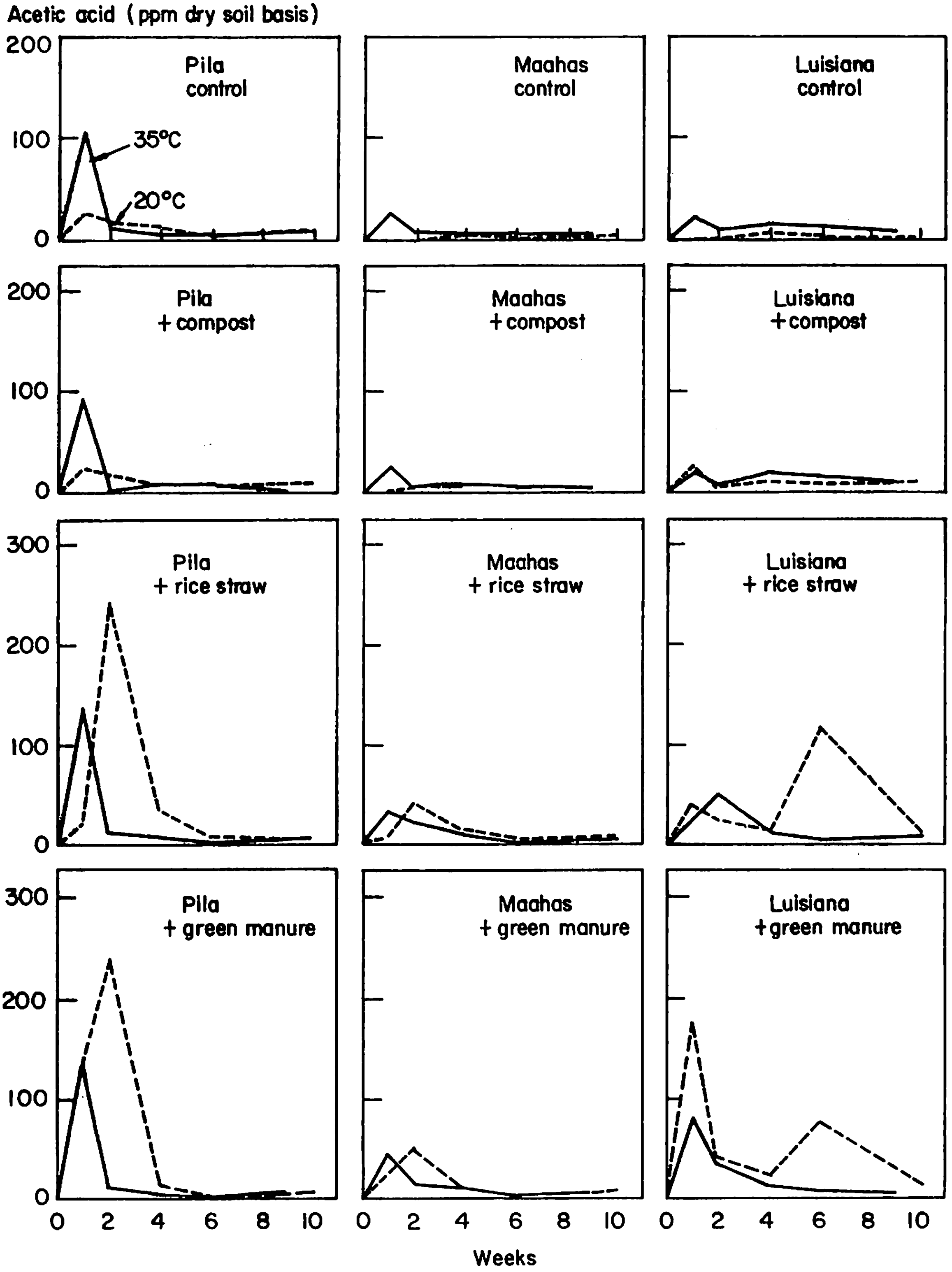

Fig. 12. Kinetics of acetic acid in submerged soils as affected by temperature and added organic materials

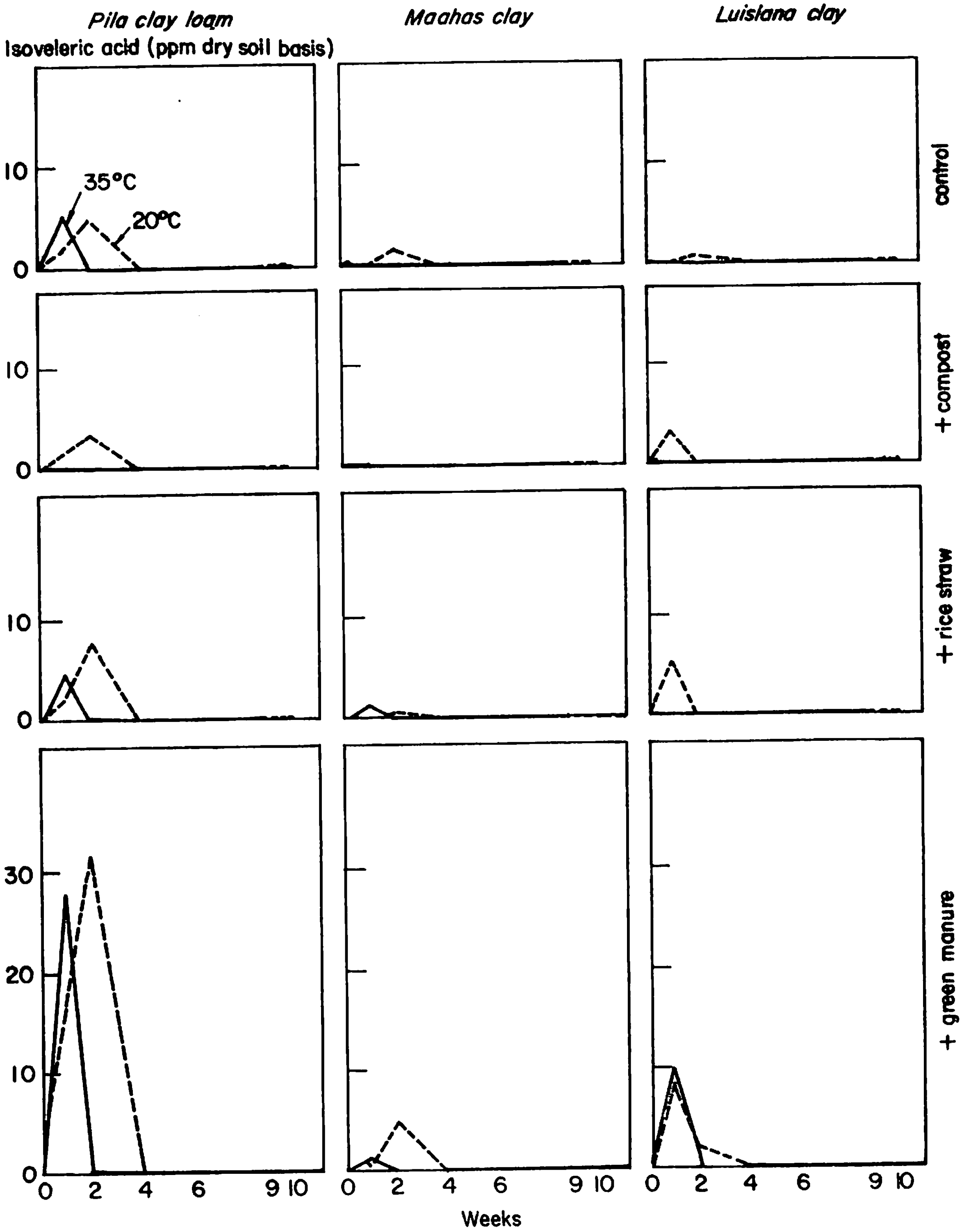

Fig. 13. Kinetics of isovaleric acid in submerged soils as affected by temperature and added organic materials

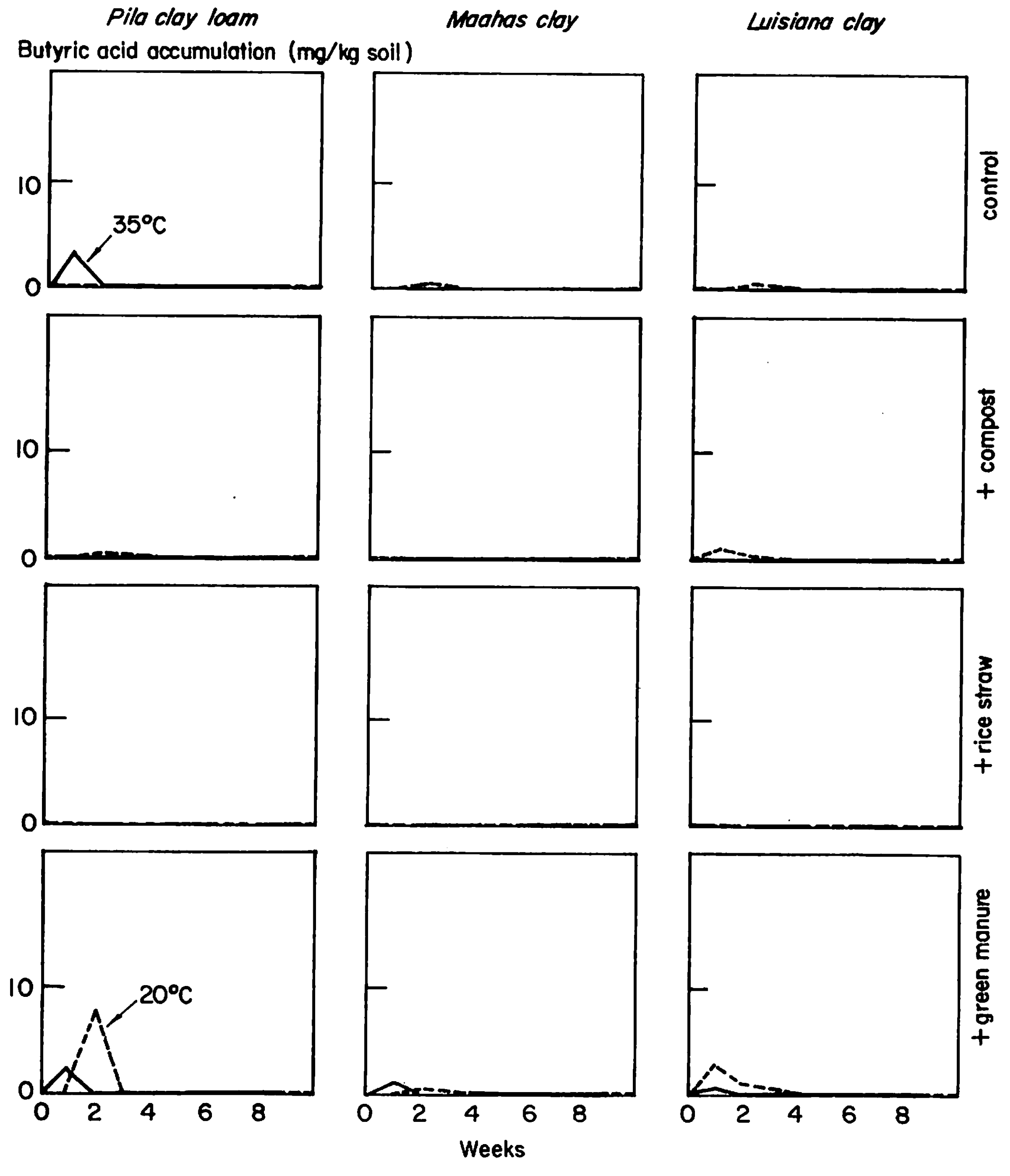

Fig. 14. Kinetics of butyric acid in submerged soils as affected by temperature and added organic materials

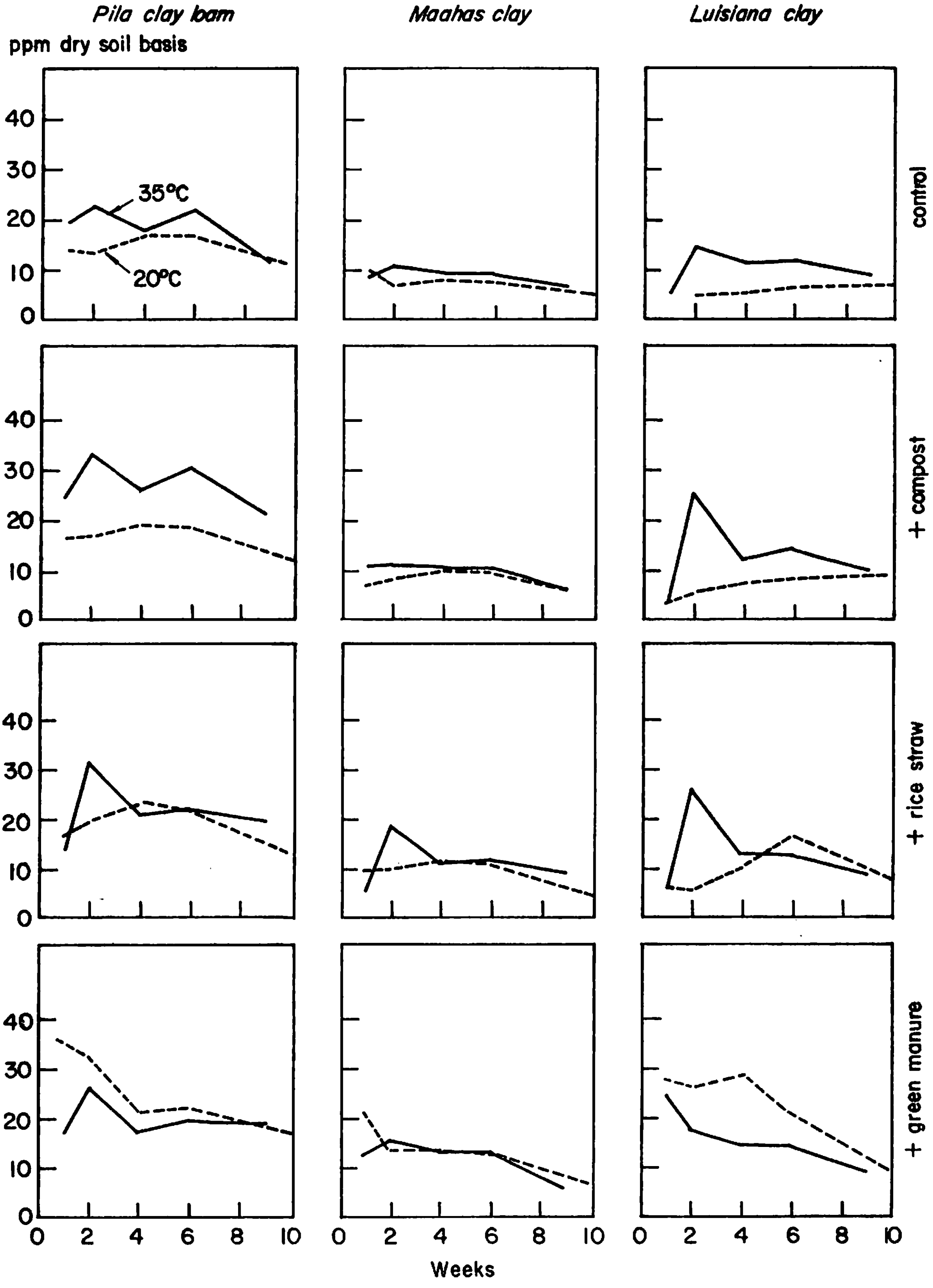

Fig. 15. Kinetics of organic reducing substance in the water extract of submerged soils (calibrated by p-hydroxybenzoic acid)