Mineralization and Changes in Microbial Biomass in Water-Saturated Soil Amended with Some Tropical Plant Residues

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Received March 29, 1995; accepted in revised form November 29, 1995

An incubation experiment was conducted in the laboratory at 25 and 35°C during 56 d to analyze the mineralization patterns and the changes in microbial biomass in water-saturated soils amended with 6 types of organic materials (O.M.) including residues from 4 tropical plants. C and N mineralization in amended and non-amended soils was influenced by the temperature. A significantly positive correlation was observed between C mineralization and the amount of hexoses of the amended O.M. regardless of the period of incubation. A negative relationship between the N mineralized from amended O.M. and C/N ratios and the amounts of cellulose plus hemicellulose of the added O.M. was observed during the period of maximum mineralization on the 49th day at 25°C. The critical C/N ratio value for N mineralization and immobilization was observed in dhaincha (15.7) and cowpea (22.0).

The pattern of changes in microbial biomass C and N was almost similar at both 25 and 35°C. The amount of biomass C and N gradually increased up to a period of 28 to 42 d and thereafter decreased gradually. A significant increase in the amount of biomass C and N was observed in O.M. amended soils over the control. The contribution of rice straw and cowpea to biomass C formation was significantly larger than that of other O.M. at the end of incubation (56 d). In the case of biomass N, the contribution of rice straw was significantly larger than that of other O.M. except for azolla at 25°C and cowpea at 35°C. The significant contribution of rice straw and cowpea to biomass formation suggests that microbial biomass remaining in soil on the 56th day had been influenced by the combination of a larger amount of cellulose plus hemicellulose and higher C/N ratio in plant residues.

Key Words: **C and N mineralization, fumigation-extraction method, microbial biomass, tropical plant residues, water-saturated soil.**

Microbial biomass is considered to be a transformation agent of soil organic materials and a labile reservoir of nutrients such as C, N, P, and S (Jenkinson and Ladd 1981). Microbial biomass accounts for only a small fraction of the total amount of C and N, but it has a relatively rapid turnover (Marumoto 1990). Therefore, the amount, activity and species composition of the microbial biomass are key factors controlling the amounts of C and N mineralized.

Microbial life in soil is largely determined by environmental factors. Temperature affects the dynamics and activities of the soil microbial biomass (Campbell et al. 1973; Sarathchandra et al. 1989; Insam 1990). Moreover, the size of the microbial biomass is regulated by substrate, water availability and protection capacity of the soil (Van Veen et al. 1984; McGill et al. 1986). In addition, the decomposition of organic materials (O.M.) in soils is also affected by the quality and quantity of the O.M. and by some environmental conditions.

It is important to note that the fertility status and organic matter content of tropical soils are relatively lower than those of soils for agricultural use in the temperate region (Brady 1974; Anderson and Domsch 1980). Since the rapid decomposition of O.M. in tropical soils enhances the loss of nutrients, it is difficult to improve the fertility status of the tropical soils. However, regular addition of a sufficient amount of O.M. to soil may increase the fertility of soil or minimize the loss of nutrients. Although various kinds of plant residues are available in tropical regions for green manuring purposes, there is little information on their decomposition pattern and their effect on the changes in microbial biomass in soils under different conditions of temperature and moisture.

Recently, some reports have been published on the changes in microbial biomass in upland soil amended with various cereal straws and leguminous crops (Brookes et al. 1990; Ocio et al. 1991; Amato and Ladd 1992; Bremer and van Kessel 1992; Patra et al. 1992). In our previous study (Azmal et al. 1996) the pattern of changes in the microbial biomass and mineralization of some tropical plant residues applied in upland soil at different temperatures has been already determined. However, the pattern of changes in microbial biomass in water-saturated soil amended with tropical plant residues has not been documented. On the other hand, some information is available on the measurements of microbial biomass in waterlogged soils without addition of O.M. (Inubushi et al. 1984; Watanabe and Inubushi 1986; Inubushi and Watanabe 1987; Inubushi and Wada 1988; Inubushi et al. 1991) and with the addition of cellulose paper (Saito et al. 1990). In the present study, therefore, an attempt was made to investigate the changes in the microbial biomass and mineralization pattern in water-saturated soils amended with some tropical plant residues.

MATERIALS AND METHODS

Soil. Soil used in this study was the same as that indicated in the previous study in upland soil (Azmal et al. 1996). Physico-chemical characteristics of the soil were described in the previous report.

O.M. used. Six types of O.M., azolla (*Azolla pinnata*), water hyacinth (*Eichhornia crassipes*), dhaincha (*Sesbania rostrata*), cowpea (*Vigna unguiculata*), rice straw (*Oryza sativa*), and farmyard manure were selected on the basis of their C/N ratios out of 8 O.M. used in the previous study (Azmal et al. 1996). Among the O.M., azolla, water hyacinth, dhaincha, and cowpea are tropical plant residues. Rice straw used in this study was cultivated in Japan but basically it is a tropical cereal crop. Farmyard manure which was collected in Japan and is an organic manure is also used in tropical areas. Chemical characteristics of the O.M. were described in the previous report.

Addition of O.M. to soil and incubation. Moist soil samples equivalent to 20 g oven-dried soil were weighed in 125 mL conical flasks for measuring the amount of N mineralization and the amount of biomass C and N and in 250 mL bottles with plugs for measuring the amount of CO_2-C. Subsequently, the soils were amended with O.M. containing 40 mg C and then the moisture content of the soils was adjusted to 100% of the water holding capacity (WHC). The mouths of the flasks were closed with aluminum-foil and the 250 mL bottles were closed with plugs having two openings. The flasks and bottles containing samples were incubated up to a maximum period of 56 d at 25 and 35°C which are close to the average temperature in tropical areas. Mineralization of C and N and biomass C and N were measured intermittently. The flasks and the bottles were weighed every week and the weight loss was compensated by the addition of water to maintain a constant moisture level throughout the incubation period. Incubation was carried out in triplicate for measuring C and N mineralization and in duplicate for biomass C and N.

Measurement of C and N mineralization. The amount of CO_2 evolved during incubation was measured according to the method described by Marumoto et al. (1974) as indicated previously (Azmal et al. 1996). During the decomposition of O.M. in water saturated soil (partially anaerobic conditions), CH_4, organic acids, and dissolved CO_2 in soil water may also be produced. However, in this experiment only the amount of evolved CO_2 was measured as an index of C mineralization. Therefore, the calculated amount of C mineralization might be lower than the actual amount. However, the pattern of C mineralization may be analyzed only by CO_2 measurement. The soil inorganic N ($NH_4^+ + NO_3^-$) was extracted with 10% KCl solution (soil : KCl solution = 1 : 4) and the content was determined by a steam distillation procedure with MgO and Devardas alloy (Bremner 1965).

Measurement of biomass C and N. Reliable biomass measurements by the fumigation-extraction method have been reported in paddy (i.e. waterlogged) soils (Inubushi et al. 1989, 1991). The fumigation-extraction method (Brookes et al. 1985a, b; Vance et al. 1987) as described previously (Azmal et al. 1996) was adopted to estimate the amount of biomass C and N. In this study the amount of biomass C (B_c) and of biomass N (B_n) was calculated from the equations, $B_c = 2.64E_c$ (Vance et al. 1987) and $B_n = E_n/0.54$ (Brookes et al. 1985b), respectively.

RESULTS AND DISCUSSION

C mineralization in O.M.-amended soils

The significant effect of temperature on the decomposition of O.M. has been reported by many investigators (Jenkinson and Ayanaba 1977; Westcott and Mikkelson 1985; Joergensen et al. 1990). In this study the amount of C mineralization (CO_2-C evolution) was larger at 35°C than at 25°C (Fig. 1). Both at 25 and 35°C, flush decomposition of C occurred within the first week of incubation and thereafter the rate of mineralization became relatively slow. Among the O.M., the pattern of C mineralization in soils amended with azolla and dhaincha was similar both at 25 and 35°C and the amount of CO_2-C evolved from these two soils was larger than that from other amended soils. In contrast, the smallest amount of C mineralization was observed in the soil amended with farmyard manure. The amount of C mineralization in the soils amended with cowpea, rice straw, and water hyacinth was similar at 35°C. But at 25°C, C mineralization in the water hyacinth amended soil was lower than that in the soils amended with cowpea and rice straw.

A larger amount of CO_2-C evolved from soils amended with azolla and dhaincha, presumably because these O.M. contain larger amounts of hexoses than the other O.M. (Azmal et al. 1996). Water soluble materials, such as hexoses are first decomposed, followed by cellulose and hemicellulose, and lignin are the most resistant organic compounds in plant materials (Alexander 1977). The smallest amount of CO_2-C evolved from the farmyard manure treated soil was probably due to the fact that the easily decomposable portion of

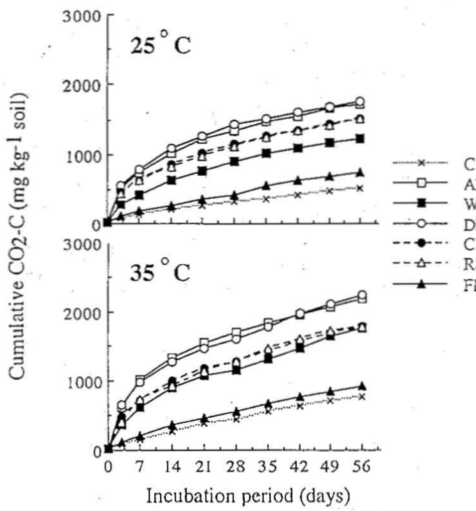

Fig. 1. C mineralization patterns in water-saturated soils amended with various organic materials at 25 and 35°C. C, control; AZ, azolla; WH, water hyacinth; DH, dhaincha; CP, cowpea; RS, rice straw; FM, farmyard manure. The maximum coefficient of variation (cv) is 7.8%.

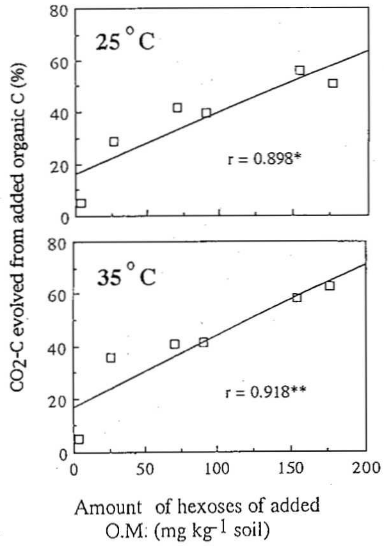

Fig. 2. Relationship between the percentage of mineralized C of added organic C and the amount of hexoses of the added organic materials after 28 d of incubation at 25 and 35°C. *, ** Significant at 5% and 1% level, respectively.

organic C in farmyard manure had been already exhausted during the composting process. The content of hexose in farmyard manure was the lowest (0.07%).

In this study, the amount of CO_2-C evolved from the added organic C at any period of incubation was significantly correlated with the amount of hexoses of the added O.M. The relationship for 28 d was selected because during this period of incubation CO_2-evolution almost reached a steady state (Fig. 2). The report of Alexander (1977) suggested that the decomposition of organic matter increased with increasing amount of water soluble fractions in the organic matter.

N mineralization and immobilization in O.M.-amended soils

In a laboratory experiment using waterlogged soils (Nagarajah et al. 1989), the release of NH_4^+-N from different green manures increased rapidly from 2 to 4 weeks and thereafter it remained nearly constant for 8 to 12 weeks. Ishikawa (1988) reported that N mineralization of green manure in paddy soil reached maximum values within 4 to 6 weeks at 23–30°C. In this study the amount of inorganic N ($NH_4^+ + NO_3^-$) accumulated in the O.M.-amended soils was larger at 35°C than at 25°C (Fig. 3). The release of N proceeded gradually and the amount of inorganic N reached a maximum value on the 49th day at 25°C and on the 28th to 42nd d at 35°C.

Figure 4 illustrates the patterns of N mineralization and immobilization which were calculated by subtracting the amount of inorganic N released in control soil from that in amended soils. Among the O.M., immobilization was observed for rice straw (C/N ratio, 60.0) throughout the incubation period both at 25 and 35°C. In contrast, only mineralization was observed in soils treated with azolla, water hyacinth, and farmyard manure (C/N ratios

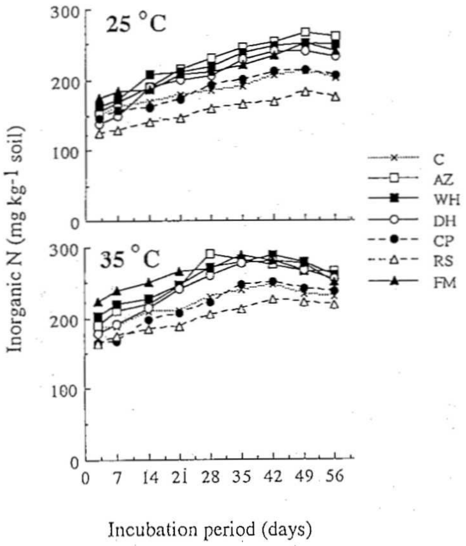

Fig. 3. N ($NH_4^+ + NO_3^-$) release pattern in water-saturated soils amended with various organic materials at 25 and 35°C. C, control; AZ, azolla; WH, water hyacinth; DH, dhaincha; CP, cowpea; RS, rice straw; FM, farmyard manure. The maximum cv is 5.6%.

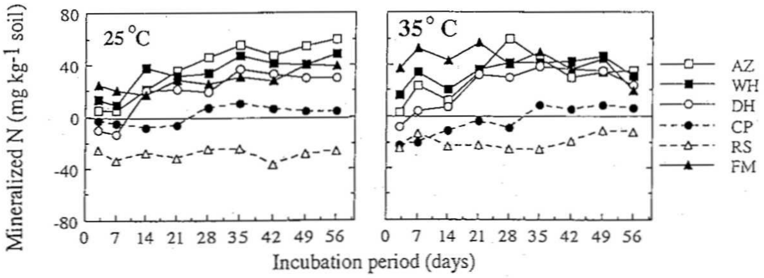

Fig. 4. N mineralization and immobilization patterns of organic materials used for amendment in water-saturated soils at 25 and 35°C. The values shown in the figure were obtained by subtracting the amount of inorganic N in control soil from that in amended soils. Positive and negative values indicate mineralized and immobilized N, respectively. C, control; AZ, azolla; WH, water hyacinth; DH, dhaincha; CP, cowpea; RS, rice straw; FM, farmyard manure.

below 15.0) throughout the incubation period. The simultaneous occurrence of mineralization and immobilization for dhaincha and cowpea revealed that their C/N ratios (15.7–22.0) were in the range of the critical value of the C/N ratio for mineralization and immobilization. Black (1968) reported that in many cases, the critical C/N ratio for N mineralization and immobilization ranged between 15 and 33 values which are in good agreement with the critical value observed in this study.

The relationships between the amount of N mineralized from the O.M. used for amendment and the C/N ratios and the amounts of cellulose plus hemicellulose of the added O.M. which were determined during the period of maximum mineralization on the 49th day at 25°C were negative (Fig. 5). In the case of 35°C, since maximum mineralization was observed on different days (28, 35, and 42 d), the relationship was not examined. The negative relationships between N mineralization and C/N ratios of the O.M. under both aerobic and anaerobic conditions of soils were reported by many investigators (Hirose 1973; Weeraratna 1979; Frankenberger and Abdelmagid 1985; Wagger 1989).

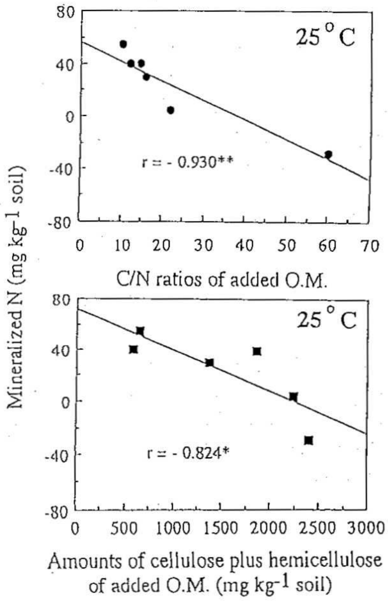

Fig. 5. Relationship between the amount of mineralized N and C/N ratios and the amounts of cellulose plus hemicellulose of the added organic materials at the period of maximum mineralization observed after 49 d at 25°C. The values shown in the figure were obtained by subtracting the amount of inorganic N in the control soil from that in amended soils. *, ** Significant at 5% and 1% level, respectively.

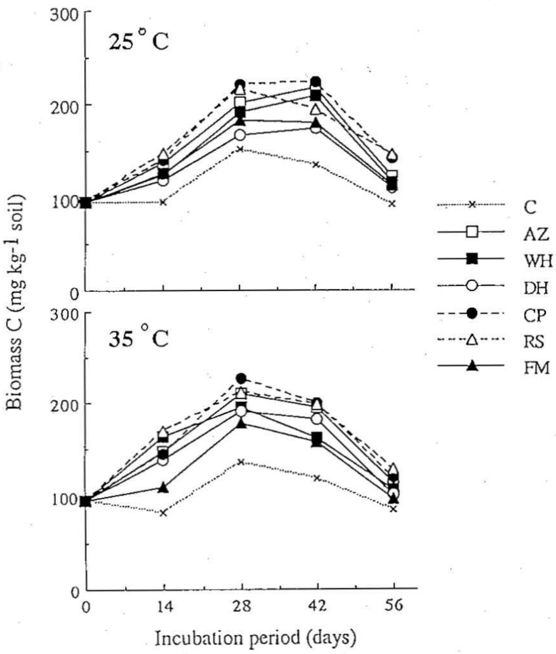

Fig. 6. Changes in microbial biomass C in water-saturated soils amended with various organic materials at 25 and 35°C. C, control; AZ, azolla; WH, water hyacinth; DH, dhaincha; CP, cowpea; RS, rice straw; FM, farmyard manure. The maximum cv is 7.0%.

In our previous report (Azmal et al. 1996) C and N mineralization was measured in the upland soil at 60% WHC, where the amount of C and N mineralization was larger than that observed in this study. The rapid decomposition of the O.M. in upland soil was probably promoted by the higher activity of the microbial decomposers in the presence of optimum moisture (60% of WHC) and available oxygen in soil. However, negative relationships were observed between the N mineralization and C/N ratios of the added O.M. in both water saturated and upland soils. In addition, the relationships between the mineralized N and the amounts of cellulose plus hemicellulose of the added O.M. were also negative in both soils.

Changes in biomass C and N

The pattern of changes in microbial biomass C and N was almost similar at both 25 and 35°C (Figs. 6 and 7). The amount of biomass C and N gradually increased up to a period of 28 to 42 d of incubation and thereafter gradually decreased. On the other hand, the amount of biomass C and N increased rapidly within a week at both 25 and 35°C in the upland soil with 60% WHC and thereafter decreased immediately (Azmal et al. 1996).

The pattern of changes in the microbial biomass observed in this study in water-saturated soil differed from that observed in the previous study in upland soil with 60% WHC. Moreover, the amount of maximum biomass C and N formation in water-saturated soils was significantly smaller than that in upland soils. The period of maximum biomass formation was longer (28 d) in water-saturated soil compared to that in the upland soil (7 d). In the previous study, in upland soil (Azmal et al. 1996) the maximum amount of biomass C and N formed in the O.M.-amended soils at 25°C ranged from 315 to 400 mg kg^{-1} soil and 75 to 93 mg kg^{-1} soil, respectively; and at 35°C it ranged from 345 to 411 mg kg^{-1} soil and 78 to 90 mg kg^{-1} soil, respectively. On the other hand, in the present study the maximum amount of biomass C and N formed in the O.M.-amended soils ranged from 170 to 223 mg kg^{-1} soil and 50 to 64 mg kg^{-1} soil, respectively at 25°C; and 178 to 226 mg kg^{-1} soil and 54 to 64 mg kg^{-1} soil, respectively at 35°C. The smaller amount of biomass C and N formed in the water-saturated soil was probably due to the higher moisture content,

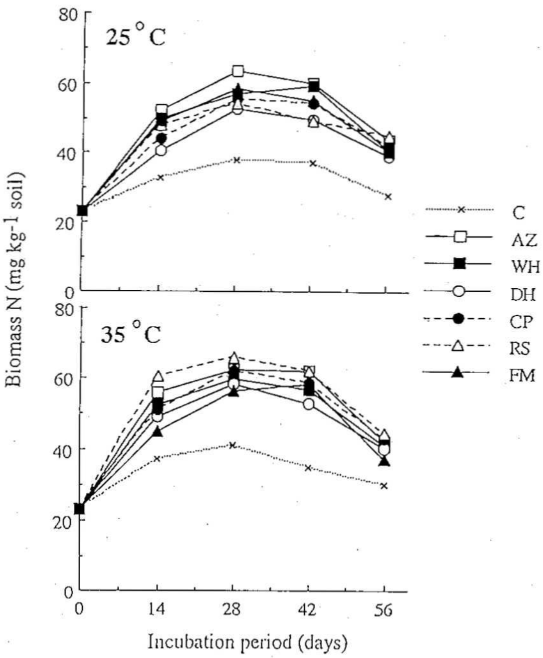

Fig. 7. Changes in microbial biomass N in water-saturated soils amended with various organic materials at 25 and 35°C. C, control; AZ, azolla; WH, water hyacinth; DH, dhaincha; CP, cowpea; RS, rice straw; FM, farmyard manure. The maximum cv is 6.3%.

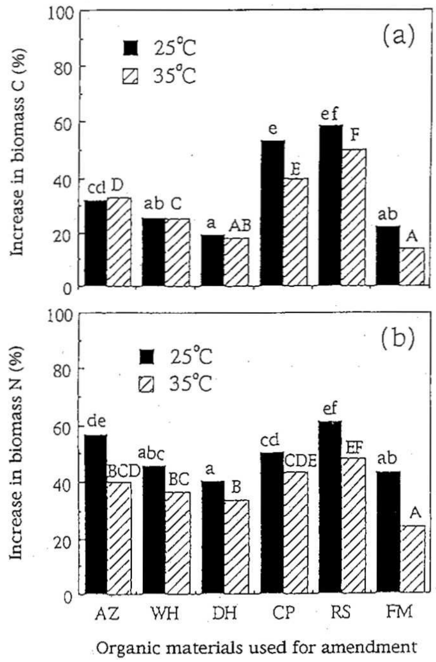

Fig. 8. Percentage of increase of (a) biomass C and (b) biomass N over control following the addition of organic materials to water saturated soils after 56 d of incubation at 25 and 35°C. Abbreviations used for the organic materials are the same as those in the Fig. 7. In (a), small letters indicate the comparison among the organic materials for 25°C and capital letters for 35°C. In (b), small letters indicate the comparison among the organic materials for 25°C and capital letters for 35°C. Common letters in the figure (a) or (b) do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

because a high soil moisture content reduces the availability of oxygen in soil, which adversely affects microbial growth and activity. Linn and Doran (1984) reported that aerobic microbial respiration increased with the increase of the soil water content and reached a maximum value at 60% water saturation. Aerobic microbial activity declined above 60% as additional water apparently limited the diffusion of oxygen through soil. However, Stott and Martin (1989) observed that favorable moisture conditions for optimum organic matter decomposition in soils ranged from about 50 to 90% of the WHC and above 90%, biological activity and decomposition rate were adversely affected due to the lack of O_2. The above reports suggested that the reduced growth and activity of the microbial biomass in water-saturated soil were mainly due to the limited supply of oxygen which is essential to maintain efficient metabolic processes.

Increase in biomass C and N following the addition of O.M.

The amount of biomass C and N in the O.M.-amended soils increased significantly over the control. The percentage of increase in biomass C and N in the O.M.-amended soils over the control soil was calculated at the end of the incubation period (Fig. 8). The effect of O.M. on biomass formation which reflected the soil fertility can be better evaluated during the period when the biomass changes reached almost a steady state. However, the duration of this experiment was not long enough for biomass formation to reach a clear steady state. On the other hand, the amount of biomass in the control soils at both 25 and 35°C was nearly equal to the amount recorded on 0 d and that in amended soils after 56 d also became closer to the amount recorded on 0 d. Therefore, in this experiment we evaluated the effect of O.M. on biomass formation by adopting 56 d as an indication of steady state period. The increase in the amount of biomass C ranged from 19.0 to 58.6% at 25°C and 14.2 to 50.0% at 35°C. In the case of biomass N, the increase of the amount ranged from 40.5 to 61.3% at 25°C and 24.3 to 48.3% at 35°C. These variations in the increase in the amount of biomass C and N after 56 d may be affected by the quality of O.M. Regarding biomass C and N formation, most of the plant residues showed a significantly higher performance than farmyard manure. The increase in the amount of biomass C in the soils amended with rice straw and cowpea was considerably larger compared to that with other plant residues, and in the azolla treated soil the amount of biomass C was significantly larger than in the case of water hyacinth at 35°C and dhaincha at both 25 and 35°C. However, biomass N formation was larger when rice straw was used compared to other O.M. except for azolla at 25°C and cowpea at 35°C.

The cellulose plus hemicellulose contents and C/N ratios of rice straw and cowpea were significantly higher than those of other O.M. (Azmal et al. 1996). The larger biomass formation in rice straw and cowpea after 56 d was probably influenced by these slow-release substances except for azolla. In addition, higher C/N ratio and hemicellulose content of rice straw than those of cowpea may have retarded the decline of biomass in the latter period of incubation (56 d). Paul and Clark (1989) mentioned that soluble organic substances, such as sugars, amino sugars, organic acids, and amino acids were readily leached from plant residues and were quickly utilized by soil organisms. However, the biomass C and N converted from these easily decomposable organic substances, are metabolized immediately outside of microbial bodies (Marumoto 1990). After the decomposition of soluble substances, cellulose and hemicellulose start to be decomposed. Therefore, the higher C/N ratio and cellulose plus hemicellulose contents may have both contributed to the biomass formation at the latter stage of incubation in this experiment. However, this assumption was not valid in the case of azolla. The biomass formation in azolla was larger compared to that of water hyacinth and dhaincha although the cellulose and hemicellulose contents in azolla were significantly lower than those in water hyacinth and dhaincha. The cause of the larger contribution of azolla to biomass formation is not clear. The lower contribution of farmyard manure to the increase in biomass is probably due to the presence of highly undecomposable organic fractions which were resistant to microbial attack.

Acknowledgments. We thank Dr. B. Kanke, Agricultural College of Fukushima Pref. for his assistance in the analysis of some saccharides of the organic materials used.

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