Mineralization and Microbial Biomass Formation in Upland Soil Amended with Some Tropical Plant Residues at Different Temperatures

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A model experiment was carried out at 15, 25, and 35°C to investigate the changes in microbial biomass and the pattern of mineralization in upland soil during 8 weeks following the addition of 8 organic materials including 6 tropical plant residues, ipil ipil (*Leucaena leucocephala*), azolla (*Azolla pinnata*), water hyacinth (*Eichhornia crassipes*), dhaincha (*Sesbania rostrata*), cowpea (*Vigna unguiculata*), and sunhemp (*Crotalaria juncea*). The amounts of CO_2-C evolved and inorganic N produced at 35°C were about 2 times larger than those at 15°C. At any temperature, the flush decomposition of C was observed within the first week and thereafter the rate of mineralization became relatively slow. A negative correlation was observed between inorganic N and C/N ratios of the added organic materials. The relationships between the amounts of cellulose or cellulose plus hemicellulose and the amount of mineralized N of the added organic materials were also negative.

The changes in the microbial biomass were affected by temperatures. The amount of biomass C and N was maximum after 42 d of incubation at 15°C, and after 7 d at 25 and 35°C, and thereafter decreased. The rate of biomass decline was slower at 15°C and faster at 35°C than at 25°C. Regardless of the temperatures, the addition of organic materials enhanced microbial biomass formation throughout the incubation periods.

Key Words: **chloroform fumigation-extraction, immobilization, microbial biomass, mineralization, tropical plant residues.**

In most soils, living organisms (soil microbial biomass) make up only 1–3% of total organic matter (Brookes et al. 1990). Nevertheless, the soil microbial biomass exerts an important influence on soil carbon and nutrient cycling, both through the oxidation of soil organic matter and the storage of carbon and mineral nutrients (Anderson and Domsch 1980).

Crop productivity of soil depends mainly on the amount of available nutrients in soil and significant amounts of these available nutrients are derived from soil microbial biomass. A part of the microbial population normally dies due to the changes in the environmental conditions (Marumoto et al. 1982). These dead cells can easily be decomposed and mineralized by the surviving microorganisms (Jenkinson 1976; Anderson and Domsch 1978; Nelson et al. 1979) and they supply a considerable amount of nutrients for the growing plants (Anderson and Domsch 1980; Marumoto 1984; Inubushi and Watanabe 1986). The analysis of the dynamics of the biomass fraction in soil is, therefore, essential for the maintenance and improvement of soil fertility.

Organic materials added to soils supply energy through their decomposition and stimulate microbial activity for the multiplication of their cell number or the increase of their population. Thus organic materials exert a considerable effect on the formation of microbial biomass. Moreover, the decomposition of organic materials and the activity of microbial biomass in soil are affected by the quality and quantity of added organic materials and by some environmental conditions such as temperature and moisture. Although there are some reports on the mineralization pattern of several organic materials, the information on the relationship between the mineralization pattern and the chemical properties of tropical plant residues is very limited. Furthermore, there is very little information on the pattern of changes in microbial biomass in soil amended with tropical organic materials at different temperatures. Therefore, a model experiment was conducted with a view to investigate the changes in microbial biomass C and N and the mineralization pattern in upland soils amended with various tropical plant residues at different temperatures.

MATERIALS AND METHODS

Soil. Soil samples were collected from an upland field of Yamaguchi University Farm at 0–15 cm depth, partially air-dried, and passed through a 2-mm mesh sieve. The moist soil samples were kept in polythene bags and preincubated for 3 weeks at 20°C in order to remove the effect of handling on soil respiration (Rovira and Greacen 1957). The physico-chemical characteristics of the soil are listed in Table 1.

Organic materials used. Eight organic materials (O.M.) listed in Table 2 were used in this experiment. Rice straw and farmyard manure were collected from Japan and the other 6 tropical plant residues from Bangladesh. The O.M. were dried, ground, and passed through a 2-mm mesh sieve. The chemical characteristics of the O.M. are shown in Table 2.

Addition of organic materials to soil and incubation. Moist soil samples equivalent to 20 g oven-dried soil were weighed in 125 mL flasks for measuring N mineralization and biomass C and N; and in 250 mL bottles with plugs for measuring CO_2-C. Subsequently, the soils were amended with ground organic materials containing 40 mg C and the moisture content of the soils was adjusted to 60% of maximum water holding capacity (MWHC). The

Table 1. Physico-chemical characteristics of soil used.

Particle size analysis:	
% sand	53.0
% silt	26.5
% clay	20.5
Texture	Clay loam
pH(H_2O)	5.6
Organic carbon (g kg^{-1})	22.0
Total N (g kg^{-1})	2.1
C/N ratio	10.5
Mineral N (mg kg^{-1})	18.0
CEC (cmol(+) kg^{-1})	10.5
Biomass-C (mg kg^{-1})	109.0
Biomass-N (mg kg^{-1})	28.0

Table 2. Chemical properties of the organic materials (O.M.) used.

Name of O.M.[a]	T-C ($g\ kg^{-1}$)	T-N ($g\ kg^{-1}$)	C/N ratio	Hexoses[b] ($g\ kg^{-1}$)	Hemicellulose[b] ($g\ kg^{-1}$)	Cellulose[b] ($g\ kg^{-1}$)
Ipil ipil (*Leucaena leucocephala*)	406	45.6	8.9	25.4	101	62
Azolla (*Azolla pinnata*)	340	33.2	10.2	30.0	81	30
Water hyacinth (*Eichhornia crassipes*)	274	22.8	12.0	3.6	129	126
Dhaincha (*Sesbania rostrata*)	428	27.2	15.7	30.1	154	140
Cowpea (*Vigna unguiculata*)	401	18.3	22.0	14.1	156	293
Sunhemp (*Crotalaria juncea*)	435	19.2	22.7	6.9	141	281
Rice straw (*Oryza sativa*)	420	7.0	60.0	19.1	233	272
Farmyard manure	354	24.5	14.5	0.7	28	76

[a] Scientific name of O.M. is indicated in parenthesis. [b] These organic components were analyzed by the method of Kanke (1987).

mouths of the 125 mL flasks were closed with aluminum foil and the 250 mL bottles were closed with plugs having two openings. The flasks and bottles containing the samples were incubated up to a maximum period of 56 d at 15, 25, and 35°C. Mineralization of C and N and biomass C and N were measured periodically. The flasks and the bottles were weighed every week and the weight loss was supplemented by the addition of water to maintain a constant moisture level throughout the incubation period. For each treatment, incubation was carried out in triplicate.

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). In this method CO_2 evolved was absorbed by Sodatalc (Merck Co.) in two coupling U-tubes connected with an incubation bottle for 10 min and then the amount of CO_2 was obtained from the increment of U-tube weight. After the measurement of CO_2, the samples were returned to the incubator; the same samples were used every week for the measurements in a similar way. For measuring N mineralization the samples in the flasks were extracted with 10% KCl solution (soil : KCl solution = 1 : 4) and the amount of inorganic N ($NH_4^+ + NO_3^-$) was determined by the steam distillation procedure with MgO and Devardas alloy (Bremner 1965). The amount of mineralized N of added O.M. were calculated by subtracting the amount of inorganic N in the control soil from that in the amended soil.

Measurement of biomass C and N. The chloroform fumigation-extraction method was adopted to estimate the amount of microbial biomass C and N in soils. Fumigation was performed with alcohol-free chloroform for 1 d under dark condition. Fumigated and non-fumigated soils were extracted with 0.5 M K_2SO_4 for 30 min. From the same extracts the amount of biomass C and N was measured according to the method described by Vance et al. (1987) and Brookes et al. (1985a, b), respectively. The amount of biomass C (B_c) was calculated from the equation, $B_c = 2.64E_c$, where E_c is the difference between the amount of C extracted from the fumigated and non-fumigated samples. On the other hand, the amount of biomass N (B_n) was calculated from the equation, $B_n = E_n/0.54$, where E_n is the difference between the amount of N extracted from the fumigated and non-fumigated samples.

RESULTS

Mineralization of C and N in soil amended with organic materials

a. Effect of temperature. Mineralization of C in amended and unamended soils increased with increasing temperature (Fig. 1). The amount of CO_2-C produced at 35°C was more than twice that produced at 15°C. At any temperature the flush decomposition of C occurred within the first week of incubation and thereafter the rate of mineralization became relatively slow.

Similarly, the largest amount of inorganic N was produced at 35°C, followed by 25 and 15°C (Fig. 2). At 15°C, the mineralization pattern was irregular. At 35°C, the amount of inorganic N in amended soils increased gradually up to 28 to 35 d of incubation and then decreased slowly. At 25°C, the mineralization in soils amended with O.M. with lower C/N ratios (C/N$<$16) became maximum after a 28-d period of incubation, whereas in soils amended with O.M. having C/N ratios greater than 20 the level of inorganic N was almost constant after 14 d.

b. Effect of C/N ratio. The ipil ipil with the lowest C/N ratio (8.9) showed the highest C mineralization and the rice straw with the highest C/N ratio (60) showed the lowest rate of C mineralization except for dhaincha and farmyard manure (Fig. 1). The rate of C mineralization of dhaincha (C/N ratio, 15.7) was larger than that of ipil ipil. On the other hand, in the case of farmyard manure (C/N ratio, 14.5) the rate of C mineralization was very low compared to rice straw and it was almost similar to that of the control soil.

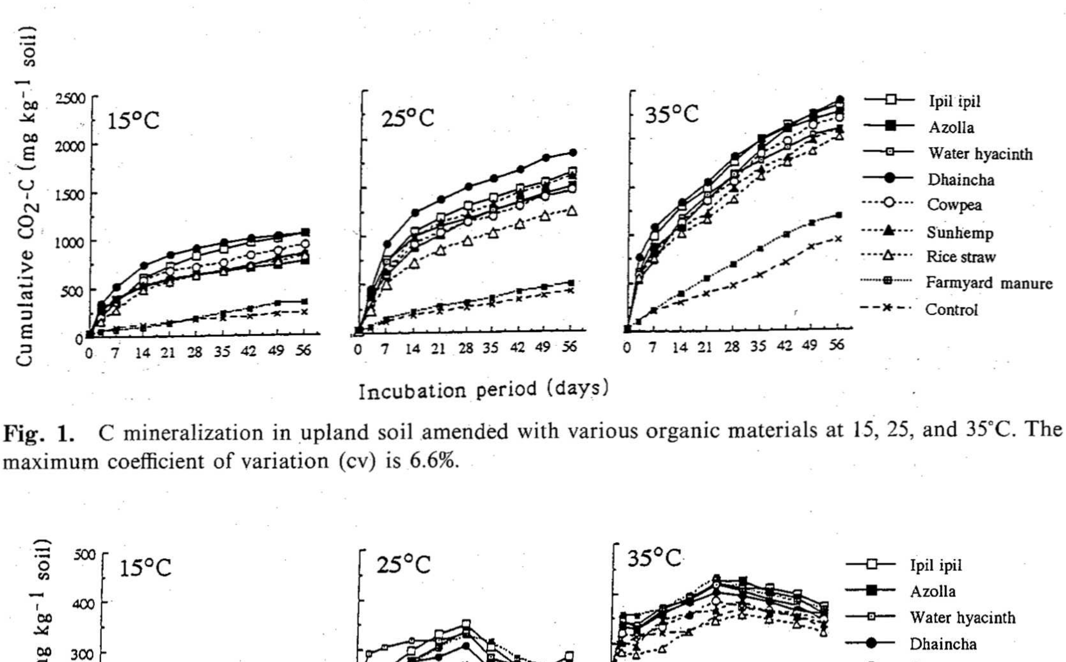

Fig. 1. C mineralization in upland soil amended with various organic materials at 15, 25, and 35°C. The maximum coefficient of variation (cv) is 6.6%.

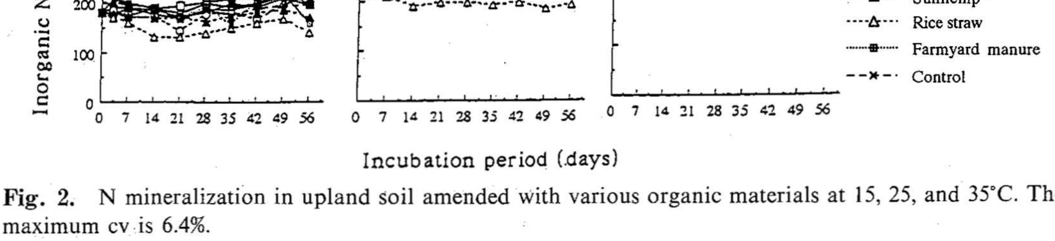

Fig. 2. N mineralization in upland soil amended with various organic materials at 15, 25, and 35°C. The maximum cv is 6.4%.

In most cases, O.M. with lower C/N ratios showed a higher rate of N mineralization and vice versa. The mineralization and immobilization patterns of 3 selected O.M. (rice straw, cowpea, and ipil ipil) based on their C/N ratios are shown in Fig. 3. In the case of rice straw the mineralized N was mostly immobilized throughout the incubation period at any temperature. At 15°C, in ipil ipil, the mineralized N was immobilized up to 14 d of incubation and in cowpea, the mineralized N was immobilized up to 42 d. At 25°C, in cowpea the immobilization was observed after 7 d and continued up to 28 d, but the immobilization was not observed in ipil ipil. At 35°C, all the O.M. except for rice straw showed N mineralization throughout the incubation periods. An almost similar pattern of

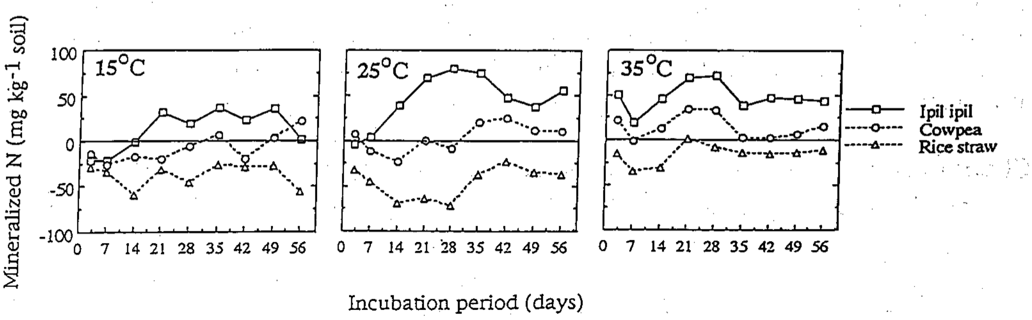

Fig. 3. N mineralization and immobilization pattern of 3 organic materials, ipil ipil, cowpea, and rice straw having C/N ratios of 8.9, 22, and 60, respectively, at 15, 25, and 35°C.

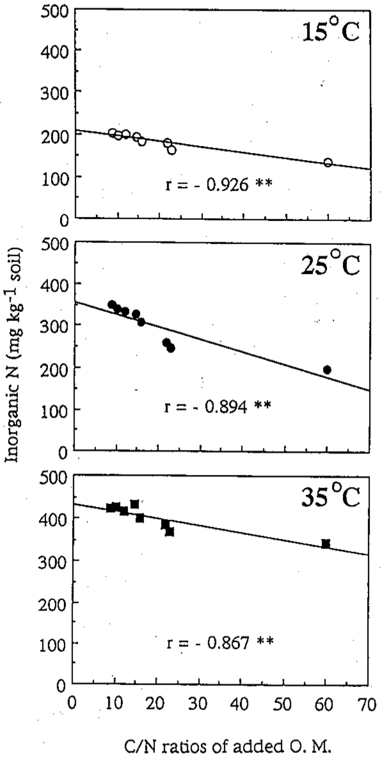

Fig. 4. Relationships between the amount of inorganic N in amended soils and C/N ratios of the added organic materials after 28 d of incubation at 15, 25, and 35°C. ** Significant at 1% level.

mineralization and immobilization was observed in soils treated with O.M. having identical C/N ratios. The correlation between the N mineralization in O.M. amended soils and the C/N ratios of O.M. was analyzed after 28 d of incubation. Figure 4 shows that inorganic N was negatively correlated with the C/N ratios of the O.M. and their correlation was highly significant ($r=-0.926^{**}$, -0.894^{**}, and -0.867^{**} at 15, 25, and 35°C, respectively).

Effect of cellulose and cellulose plus hemicellulose of the added O.M. on N mineralization

Figure 5 shows that the negative correlation between the amount of mineralized N of the added O.M. after 28 d of incubation and the amount of cellulose of the added O.M. was highly significant at 25 and 35°C ($r=-0.851^{**}$ and -0.840^{**}, respectively). Figure 6 shows that the negative relationship between the amount of cellulose plus hemicellulose of the added O.M. and the amount of N mineralized after 28 d of incubation at 25 and 35°C was also significant ($r=-0.819^{*}$ and -0.851^{**}, respectively).

Changes in microbial biomass at different temperatures

At any temperature, the addition of O.M. to soil resulted in the increase in the amount

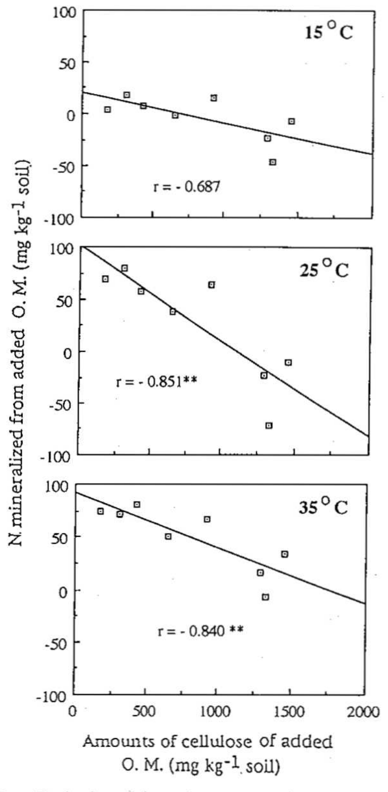

Fig. 5. Relationships between the amount of mineralized N and the amount of cellulose of the added organic materials after 28 d of incubation at 15, 25, and 35°C. ** Significant at 1% level.

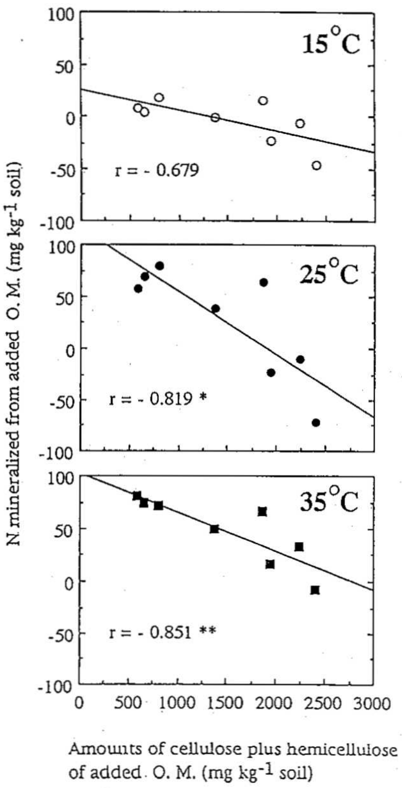

Fig. 6. Relationships between the mineralized N and the amount of cellulose plus hemicellulose of the added organic materials after 28 d of incubation at 15, 25, and 35°C. *, ** Significant at 5% and 1% level, respectively.

of microbial biomass C and N. A similar pattern of change was observed for biomass C and N. However, the pattern of change in microbial biomass C and N varied with the temperature (Figs. 7 and 8). At a low temperature (15°C), the amount of biomass C and N in all the treatments increased slowly up to 42 d of incubation except for biomass C in the control soil during the initial 14-d period and then decreased slowly. At high temperatures (25 and 35°C), however, the amount of biomass C and N in all the treatments increased rapidly within 7 d and decreased immediately and then reached a constant level after 28 or 42 d of incubation. The duration of the period of incubation required for attaining maximum biomass formation was longer at 15°C than that at 25°C or 35°C. After reaching the maximum amount, the decline of biomass was slower at 15°C and faster at 35°C than that at 25°C. However, the maximum amount of biomass C and N did not differ appreciably among 15, 25, and 35°C.

Maximum biomass N formation

Soil amended with O.M. enhanced biomass formation (Figs. 7 and 8). Increase of the

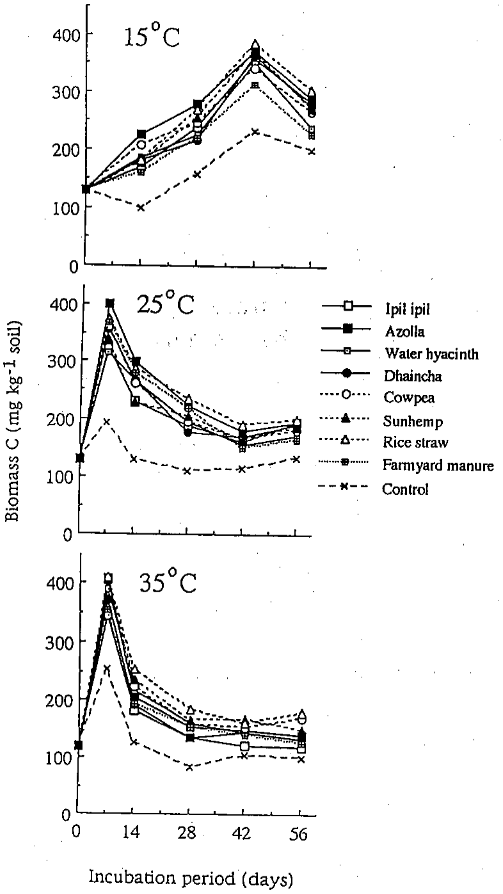

Fig. 7. Changes in microbial biomass C in upland soil amended with various organic materials at 15, 25, and 35°C. The maximum cv is 7.4%.

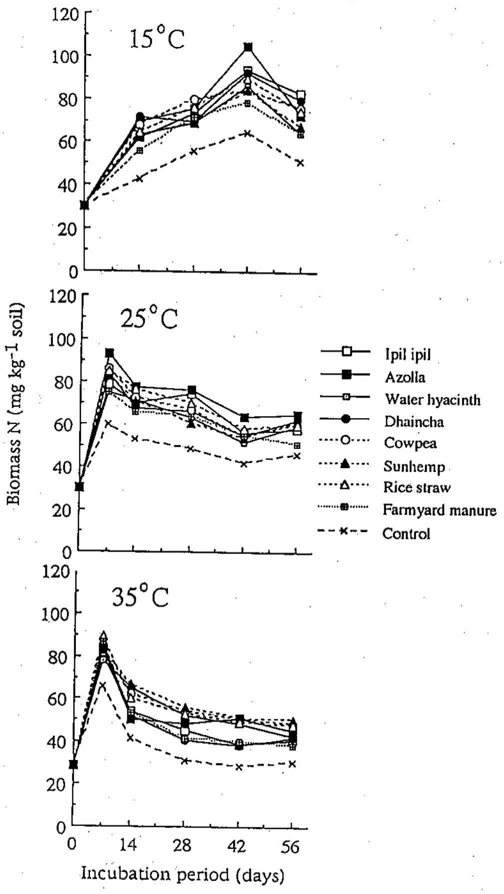

Fig. 8. Changes in microbial biomass N in upland soil amended with various organic materials at 15, 25, and 35°C. The maximum cv is 7.1%.

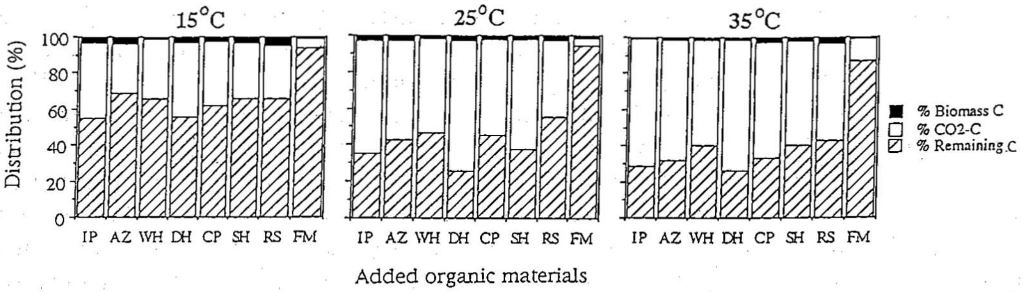

Fig. 9. Apparent distribution of the added organic C in upland soil after 56 d of incubation at 15, 25, and 35°C. IP, ipil ipil; AZ, azolla; WH, water hyacinth; DH, dhaincha; CP, cowpea; SH, sunhemp; RS, rice straw; FM, farmyard manure.

amount of biomass N by the addition of O.M. at the time of maximum biomass formation was in the range of 13.6–39.8 mg N kg^{-1} soil at 15°C, 15.0–33.0 mg N kg^{-1} soil at 25°C, and 11.8–23.4 mg N kg^{-1} soil at 35°C. The increase of the amount of biomass N at the time of maximum biomass formation showed a significant correlation with the amount of hexoses in added O.M. at 15°C ($r=0.915^{**}$), while no correlation was observed at 25 and 35°C. At the time of maximum biomass formation, the apparent percentage of N assimilated by microbial biomass from the added O.M. was 75.0% for rice straw, 21.1% for cowpea, and 12.8% for ipil ipil at 15°C; 75.0% for rice straw, 28.6% for cowpea, and 8.5% for ipil ipil at 25°C; 70.2% for rice straw, 17.3% for cowpea, and 6.2% for ipil ipil at 35°C.

Distribution of added organic C

Apparent distribution percentage of added organic C to CO_2-C and biomass C after 56 d of incubation at different temperatures is shown in Fig. 9. The percentages of CO_2-C and biomass C of added organic C were calculated by dividing the subtracted value of CO_2-C or biomass C in the control soil from that of the amended soil by the amount of added organic C. The percentage of evolved CO_2-C from added organic C was larger at higher temperatures (35°C>25°C>15°C). The reverse was true for the percentage of remaining C in the soils. During this incubation period the percentage of biomass C was slightly higher at a lower temperature.

DISCUSSION

Mineralization of C and N in O.M. amended soil. Temperature is one of the most important factors regulating the rate of organic matter decomposition in soil. The positive effect of temperature on the mineralization rate of O.M. has been reported by many investigators (Parker and Larson 1962; Alexander 1965; Scarsbrook 1965; Jenkinson and Ayanaba 1977; Joergensen et al. 1990). In this study C and N mineralization in amended and unamended soils was also affected by the temperature (Figs. 1 and 2).

Regarding the pattern of N mineralization of some green manuring crops in rice soil, N accumulation reached a maximum value within 4–6 weeks of incubation at 23–30°C (Ishikawa 1988). In our results, most of the O.M. showed maximum mineralization after 28 d at 25 and 35°C.

In this study the N mineralization-immobilization patterns of the added O.M. depended on their C/N ratios (Fig. 2). Similar relationships between C/N ratios and N mineralization-immobilization of added O.M. were reported by Hirose (1973) and Aoyama and Nozawa (1993).

A negative relationship between the N mineralization and C/N ratios, cellulose, and cellulose plus hemicellulose contents of the added organic residues was reported by Hirose (1973). Similar relationships were found in the present study (Figs. 4, 5, and 6).

Changes in microbial biomass. At a low temperature (15°C), the amount of biomass increased slowly up to 42 d and thereafter decreased. At high temperatures (25 and 35°C), however, the amount of biomass increased rapidly within 7 d and thereafter decreased more rapidly at 35°C than at 25°C (Figs. 7 and 8). At 25 and 35°C the decomposition of the O.M. proceeded rapidly probably due to the higher microbial activity and the high activity of the microorganisms enhanced the assimilation of available nutrients. It may be considered that in this range of temperatures, easily decomposable organic substances were decomposed rapidly and the released nutrients were probably immediately immobilized into the microbial body. Furthermore, it is assumed that due to the rapid assimilation of nutrients a part of the organic substances was directly absorbed by the microbial biomass. Ocio et al. (1991a) reported that most of the microbial biomass N formed due to field incorporation of straw was derived from the organic N contained in the straw itself. Therefore, at high temperatures, microbial growth increased rapidly and reached the maximum level of biomass within a week. In contrast, at a low temperature (15°C), due to the low microbial activity, a part of the easily decomposable organic substances may be decomposed during the first few weeks and the rate of mineralization was slow compared to that at higher temperatures. As the microbial activity was low the released nutrients were probably slowly assimilated by the microbial biomass. As a result, the microbial growth and multiplication increased slowly and the duration of the period of maximum biomass formation became longer. This finding is in agreement with the significant correlation observed between the increased amount of biomass N and water soluble hexoses of added O.M. at 15°C after 42 d of incubation.

In this study, the maximum amount of biomass C and N formed was similar at any temperature probably because the amount of initially available nutrients in added O.M. was identical.

The decrease of the amount of biomass was slower at 15°C and faster at 35°C than at 25°C (Figs. 7 and 8). Regarding the decline rate of biomass C a similar trend was obtained in a study on the changes in microbial biomass in grassland soils at different temperatures carried out by Joergensen et al. (1990). The decline of biomass was caused by the death of microorganisms probably due to some unfavorable conditions such as lack of available nutrients. Marumoto et al. (1982) reported that a part of microbial biomass normally dies due to the changes of the environmental conditions. Joergensen et al. (1990) also reported a general trend of death rate of microorganisms which was similar to the decline rate of biomass C.

Harmsen and Kolenbrander (1965) reported that mineralized N can be used for the formation of microbial protoplasm and if the C/N ratio of the substrate is high, all mineralized N will be absorbed by the microorganisms. Similarly, in this study, a higher percentage of N was apparently assimilated by the microbial biomass from the O.M. with higher C/N ratios. For example, at the time of maximum biomass formation, 70–75% of added rice straw N was apparently transferred to the microbial biomass. However, in ^{15}N tracer study, Bremer and van Kessel (1992) and Ocio et al. (1991b) observed that maximum values of 81 and 44% of added wheat straw ^{15}N were incorporated into the microbial biomass, respectively.

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