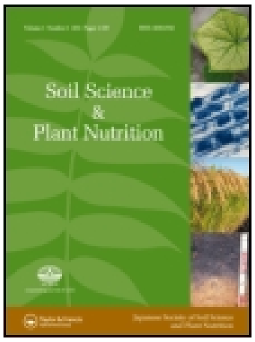

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# Decomposition and $CO_2$-C evolution of okara, sewage sludge, cow and poultry manure composts in soils

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# Decomposition and $CO_2$-C Evolution of Okara, Sewage Sludge, Cow and Poultry Manure Composts in Soils

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**A laboratory experiment was conducted to evaluate the decomposition of various composts in soils by determining the C mineralization rate and microbial biomass level. The differences in the decomposition rate of the composts were examined. The composts used consisted of okara compost made from soymilk residues, cow manure, poultry manure, and sewage sludge composts, which were applied at rates of 50 or 150 g $kg^{-1}$ soil to a Chiba Light-colour Andosol. The results showed that, in general, the amount of $CO_2$-C released increased rapidly at the initial stage, but the pattern differed among the composts used. The production of $CO_2$-C depended on the amount applied and the nature of the compost materials. The $CO_2$-C evolution from okara-treated soil was much higher than that from the soils treated with the other three composts, presumably due to the higher level of labile C as evidenced by the larger amount of microbial biomass. The value of $qCO_2$ ($CO_2$ production per unit microbial biomass) was lower in the okara compost than in the other composts. $qCO_2$ was linked to the decomposability of organic materials, reflected in the $CO_2$-C evolution.**

***Key words:*** **$CO_2$-C evolution, decomposition, metabolic quotient ($qCO_2$), microbial biomass, mineralization.**

Separate collection of organic wastes followed by composting and reuse into soil has been developed as the basic principle of wastes disposal in Japan. About 280 million tons of organic residues are generated in a year, accounting for 60% of the total solid wastes produced (Chino 2001). For sustainable agriculture, land application of organic materials is mostly practiced. The decomposition of these materials by microorganisms is a key source of supply of major plants nutrients. Organic materials added to the soils contain a wide range of C compounds that vary in their rates of decomposition. The biological breakdown of the added materials depends on the rate of degradation of each of the C-containing materials present in the sample (Gilmour et al. 1977; Reddy et al. 1980).

The cycling of soil C is essential for nutrients in soil and plant uptake, firstly, because important nutrients such as N and S become mineralized stoichiometrically with C (McGill and Cole 1981) and secondly, because available C is necessary as an energy source for heterotrophic soil microorganisms. Against this background, an incubation experiment was designed to evaluate the effects of various composts on microbial respiration and biomass in soil. The biomass supplied mainly a portion of the total amount of $CO_2$ mineralized. The rates of $CO_2$ efflux provide an indication of organic matter quality, and the conduciveness of the soil environment to the decomposition process provides conditions of moisture and temperature that are not limiting (Sparling 1997).

The decomposition of the composts (organic matter) reflects the biological availability of the soil carbon, while the release of $CO_2$ following the addition of relatively simple substrates indicates the potential carbon-mineralizing capacity of the microflora and has contributed significantly to studies on soil metabolism (Stotzky 1960; Bradley and Fyles 1995).

The okara compost was prepared from soymilk residues and sawdust, which could potentially be useful as soil amendment / organic fertilizer. In this study, the okara compost was compared with sewage sludge compost as well as with cow and poultry manure composts available on the market. The objective was to determine the degree of decomposability of the respective the composts and the amount of carbon mineralized for the increase of the C content of soils as a reflection of their quality, to utilize as a compost the large amount of okara produced each year in Japan.

## MATERIALS AND METHODS

**Incubation experiment and analytical procedures.** An incubation experiment was carried out with four different composts; the okara compost (OK) was obtained from Kikkoman Co-operation, Noda, Chiba, Japan. Sewage sludge (SS) was obtained from the Laboratory of Plant Nutrition, The University of Tokyo, while the cow manure (CM) and poultry manure (PM) composts were commercial products. The composts were dried at 65°C to determine the dry matter content. Fresh compost samples were digested with $H_2SO_4$ using Kjeldhal techniques for the determination of the total-N contents. Ammonium and nitrate in soil were extracted with 2 M KCl at 1 : 10 ratio and their contents were determined (Rowell 1994). Total C content in the soil, compost and compost-soil mixture was also determined (Rowell 1994). The pH was determined in a 1 : 10 compost: water slurry mixture (Rowell 1994). The composition of the Na, K, Mg, Ca, Al, Ni, Cu, and Zn elements in the composts was determined by ICP (ICPS-1000IV, Shimadzu, Kyoto, Japan) (Topper and Kotuby-Amacher 1990).

The soil in which the composts were incubated was a Chiba Light-colour Andosol, classified as Typic Hapludand (US Soil Taxonomy: Soil Survey Staff 1998). The soil was taken from an upland field (0–20 cm) before the application of fertilizer (Table 1). The soils were air-dried and sieved through a 2 mm mesh-sieve.

The incubation was carried out for 40 g (d.w.) of the soil samples mixed with the composts in 200 mL containers and wetted to a moisture content at 60% WHC, to ensure that microbial activities would function at their optimal rate. The amounts of composts added were 0, 2, or 6 g, corresponding to 0, 50, or 150 g $kg^{-1}$ soil fresh weight. The containers were covered tightly with a thin plastic film (Parafilm), and incubated at 30°C in the dark for 16 weeks. Every week, the moisture content was readjusted by weight to maintain a constant moisture content during the incubation period. Three replicate samples of each treatment were placed in 1-L airtight jars that contained water to maintain a moist atmosphere and a vial with 20 mL 0.3 M NaOH to absorb $CO_2$.

Excess NaOH was titrated with 0.1 M HCl after precipitation of carbonates with $BaCl_2$, and subtracted from the control without soil to determine the amount of $CO_2$ evolution from the treated soils during that week. The weekly rates of $CO_2$ evolution in the compost-treated soils were used to derive the decomposition rates of the composts. The amount of C evolved as carbon dioxide from the composting samples was calculated by subtracting the amount produced by the control soil from that produced by the compost-treated soil. Data concerning $CO_2$-C evolution of the compost samples were fitted to kinetic exponential / linear equations.

The amount of microbial biomass carbon was determined by the chloroform fumigation extraction method (Vance et al. 1987). The amount of total extractable C was measured by automated combustion (TOC 5000, Shimadzu). The biomass C level was calculated using the equation: biomass C = 2.64 $E_c$, where $E_c$ = differences in the contents of organic C of the fumigated and unfumigated samples.

**Statistical design and analyses.** A simple linear regression analysis was used to correlate the differences between the composts and their rate of decomposition. One-way analysis of variance (ANOVA), as statistical analysis, was carried out. All the analyses were performed using Sigma Stat for Windows 2.03 computing package (Jandel Corporation, Chicago, USA).

## RESULTS AND DISCUSSION

### Quality of organic manure composts

Organic by-products applied as composts contain a significant amount of proteinaceous and carbonaceous substrates, which can enrich the soil with C, N, and other nutrients. Some chemical properties of the various composts are presented in Tables 2 and 3, respectively. The pH ($H_2O$) in these composts ranged between 5.4 and 8.9 and EC from 2.2 to 6.1 dS $m^{-1}$. For the improvement of agricultural soils, compost with pH $< 7.2$ is required (Rynk et al. 1992), whereas for EC, the acceptable level for a compost is $>4.0$ dS $m^{-1}$. The pH (5.4) and EC (4.2) values in OK compared with the values in the other composts satisfied these criteria, except for SS, which had low EC values.

The C / N ratios of the composts were in the increasing order of OK < SS < PM < CM, respectively (Table

**Table 1.** Some properties of the soil.

| Soil | pH ($H_2O$) | Mineral N (g $kg^{-1}$) | | T-C (g $kg^{-1}$) | T-N (g $kg^{-1}$) | C / N ratio |
|---|---|---|---|---|---|---|
| | | $NH_4^+$-N | $NO_3^-$-N | | | |
| Chiba Light-colour Andosol* | 4.87 | 30.7 | 75.4 | 64.9 | 6.86 | 9.46 |

*Typic Hapludand (US Soil Taxonomy: Soil Survey Staff 1998).

2). The C / N ratio has been used to indicate the stability of the composts. Ratios below 15 were assumed to be indicative of a stable and mature compost. However, Mathur (1991) reported that a mature compost should have a C / N ratio of about 10 as in humus, whereas, composts with a high C / N ratio are often associated with plant phytoxicity and with the presence of volatile fatty acids. The values of C / N reported ranged from 5.8 to 8.3 (Table 2). These values satisfied the criteria outlined above. Carbon / nitrogen ratios are commonly used to predict the mineralization potential of organic materials (Azmal et al. 1996). In general, the higher the C / N ratio, the slower the mineralization. It was therefore expected in this study that the mineralization rate would be higher in the soils treated with composts with a low C / N ratio and in the soils treated with OK (C / N ratio of 5.8) than in those treated with CM (C / N ratio of 8.3).

## Carbon mineralization

Decomposition of the four composts in the soil, as determined by $CO_2$ evolution, was directly related to the amount of carbon in the compost-soil mixtures (Fig. 1). The amount of cumulative $CO_2$-C mineralized appeared to follow a first order kinetics for all the treatments. All of the compost-mixtures released $CO_2$ in relation to the total amount present, starting with a release of C during the first week of incubation, with decreasing rates thereafter. In estimating the decomposition rates of the various organic C pools in each organic material, the rates were taken from the slopes of the curves obtained from their natural logarithms.

A steep slope represents the highest decomposing rate, which was considered to be the most readily decomposable organic C fraction. The application of compost at 50 and 150 g $kg^{-1}$ rates gave values of 3.07 and 3.43 for OK, 2.93 and 2.96 for PM, 1.72 and 2.05 for CM, and 1.44 and 1.78 $\mu$g C $g^{-1}$ $week^{-1}$ for SS, respectively. Compost application at a rate of 100 g $kg^{-1}$ resulted in a higher level of $CO_2$ evolution, as indicated in all the compost-treated soils. The results showed a higher level of $CO_2$ evolution for OK than for the other composts. The amount of $CO_2$ decreased in the order of OK > PM > SS > CM. The higher level of OK may therefore suggest that higher rates of decomposition took place, which reflected the presence of available C

**Table 2.** Chemical properties of the composts used.

| Composts | pH ($H_2O$) 1 : 10 | pH (KCl) 1 : 10 | EC (dS $m^{-1}$) | Mineral N (mg $kg^{-1}$) | | T-C (g $kg^{-1}$) | T-N (g $kg^{-1}$) | C / N ratio |
|---|---|---|---|---|---|---|---|---|
| | | | | $NH_4^+$-N | $NO_3^-$-N | | | |
| Okara (OK) | 5.4 | 5.3 | 4.2 | 219 | 9.54 | 514 | 88.2 | 5.83 |
| Sewage sludge (SS) | 6.5 | ND | 2.2 | 747 | 28.14 | 288 | 44.1 | 6.53 |
| Cow manure (CM) | 6.5 | 6.3 | 3.7 | 109 | 54.84 | 157 | 18.9 | 8.31 |
| Poultry manure (PM) | 8.91 | 8.30 | 6.1 | 50.6 | 15.18 | 251 | 35.1 | 7.15 |

ND: Not determined.

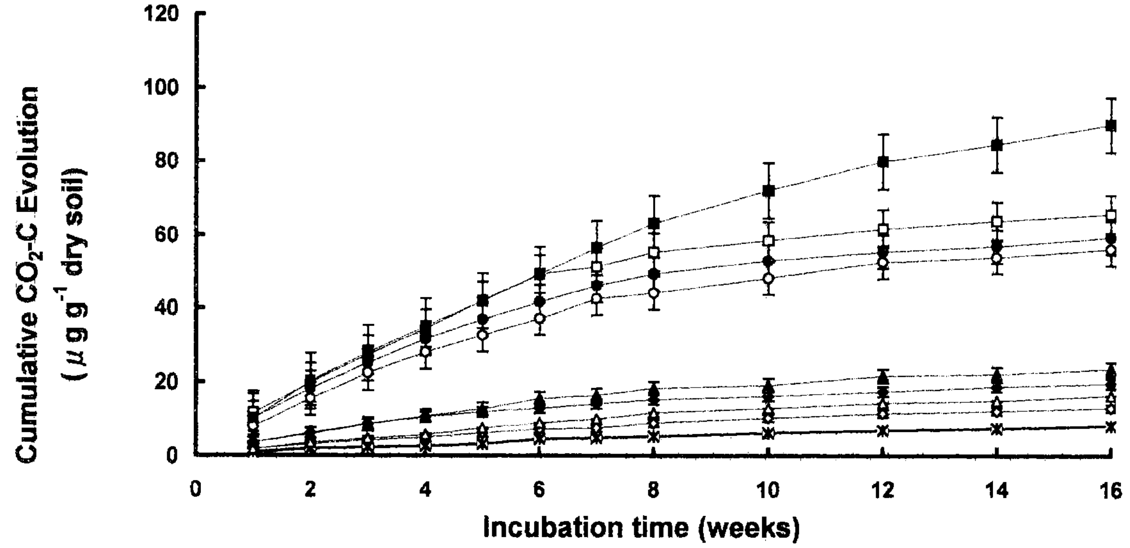

**Fig. 1.** Rates of $CO_2$ evolution from okara, cow manure, poultry manure, and sewage sludge composts incubated in soil Mean and standard error of three replicates is shown. -*- control, -□- okara compost@5%, -■- okara compost@15%, -◇- cow manure@5%, -◆- cow manure@15%, -○- poultry manure@5%, -●- poultry manure@15%, -△- sewage sludge@5%, -▲- sewage sludge@15%.

from readily decomposable compounds such as protein, as reported by Lerch et al. (1992). The lower decomposition value of CM may indicate the predominance of less decomposable substances that would, therefore, be the most resistant to microbial decomposition.

As shown in Table 3 for the Ni, Cu, and Zn contents, even though the Cd content was not reported in this study, Sadovnikova et al. (1995) indicated that the presence of heavy metals such as Cd, Ni, Cu, Pb, and Zn exerts an inhibitory effect on decomposition. For the total Ni or Cu and Zn concentrations, the acceptable concentrations for composts were <400, <1,500, and <1,800 mg kg$^{-1}$, respectively (Rao and Pandey 1996). The Cu and Zn concentrations ranged from 0.8 to 6.5 and 4.1 and 19.7 mg kg$^{-1}$, respectively whereas, that of Ni from 0.2 to 0.4 mg kg$^{-1}$ was almost negligible. These levels were far too low among the composts used (Table 3), indicating the overall suitability and quality of the composts as soil amendment.

The results of the linear regression analyses to select the optimum prediction of the amount of C mineralized during the study are presented in Fig. 2. A linear equation was drawn to represent delta ($\Delta$) $CO_2$ evolution, which is equivalent to the difference between the $CO_2$ evolution from soil treated with 50 and 150 g kg$^{-1}$ compost, as indicated by the following equations. For OK, $\Delta CO_2 = 1.54t - 1.88$; PM: $0.67t + 1.34$; SS: $0.388t + 3.17$; and CM: $0.312t + 3.11$, with a statistical difference among the composts at $p < 0.001$ (Fig. 2), where $t$ in the equation represents the time of incubation (week). The order of decomposition was OK > PM > SS > CM. To obtain more details in the plots for OK, the period 2–8 weeks was examined and it showed some irregularities; the plots were lower than the regression line, which is discussed in the next section.

The $\Delta CO_2$-C evolution represented a zero-order model if it is assumed that all the materials were undergoing decomposition at the same level (Fig. 2). Actually, this assumption may not reflect all the decomposable fractions, since the composts were made of different feedstocks and the compounds may be resistant to microbial attack, hence the use of other stepwise models might be preferable. However, the equation still supports the prediction of low rates of decomposition among the composts (Lerch et al. 1992).

These results may further be used to justify the approach of the simulation models for the C–N turnover of organic residues, where the rates of decomposition of organic C and N in soil are based on the properties of decomposing materials, C / N ratio, and other soil properties, as suggested by Van Veen et al. (1985), Molina et al. (1990), and Hadas and Portnoy (1994).

**Table 3.** Elemental composition of okara, sewage sludge, cow manure, and poultry manure composts.

| Composts / elements (mg kg$^{-1}$) | Mg | K | Ca | P | Na | Al | Ni | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|
| Okara | 31.4 | 216 | 77 | 49 | 13.2 | 5.4 | ND | 0.8 | 4.1 |
| Sewage sludge | 52.3 | 129 | 115 | 158 | 15.4 | 156.2 | 0.4 | 6.5 | 14.9 |
| Cow manure | 50.0 | 128 | 390 | 76 | 23.6 | 156.3 | 0.4 | 4.0 | 19.7 |
| Poultry manure | 55.0 | 130 | 395 | 202 | 125.3 | 20.9 | 0.2 | 2.8 | 18.6 |

ND: Not determined.

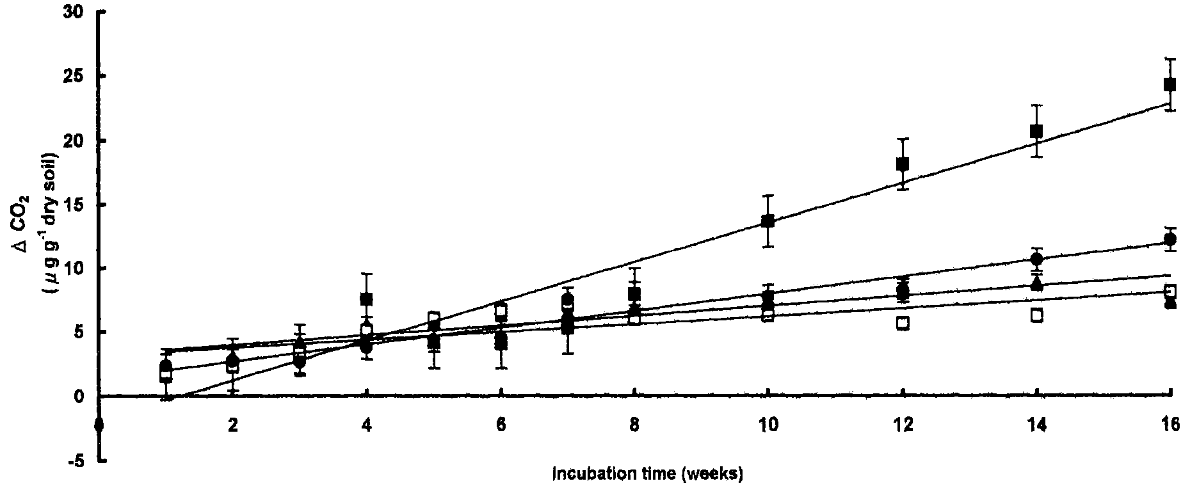

**Fig. 2.** $\Delta CO_2$ evolution with time. Mean and standard error of three replicates is shown. ■ okara (OK), ▲ sewage sludge (SS), □ cow manure (CM), ● poltry manure (PM).

## Microbial biomass

Microbial biomass has been used as an indicator of soil quality, as it responds rapidly to the changes in the soil environment that can be associated with soil contamination. The nutrients in microbial biomass are potentially available to plants (Smith and Paul 1991; Chander et al. 1995). Under favorable conditions, microbial biomass is positively correlated with the contents of organic matter, and N and P in the biomass, which are also positively correlated with N, P, and C availability (He et al. 1997).

The microbial biomass C showed similar trends for each of the composts CM, SS, PM, and OK (Fig. 3). There was a gradual increase from two weeks to about 6 weeks, followed by a general decreasing trend for PM, SS, and CM. On the other hand, OK showed a sharp rise from 2 weeks to about 8th weeks, followed by a sharp decline to the 12th week, and then a gradual decrease to the 16th week. At the end of incubation, OK contained a higher amount of accumulated biomass C than the other composts, which all showed the accumulation at almost the same level, while the accumulation was slightly higher than that of the control. Some irregularities have been observed in $\Delta CO_2$ between 2–8 weeks (Fig. 2),

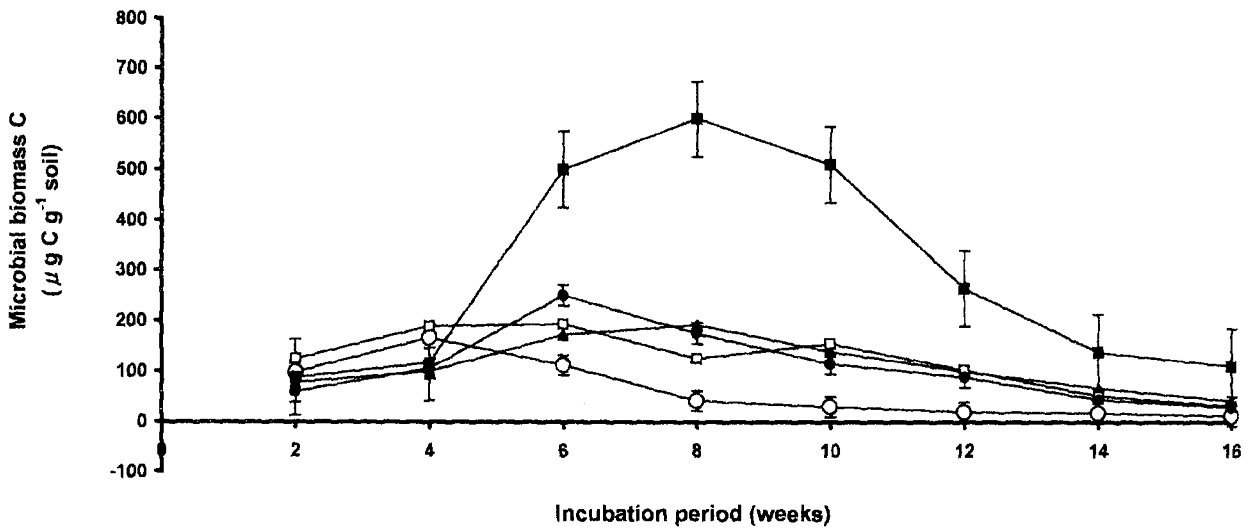

**Fig. 3.** Microbial biomass C of compost at 150 g kg$^{-1}$ application rate: Bars denote mean and SE of three replicates. -○- control, -■- okara, -▲- sewage sludge, -□- cow manure, -●- poultry manure.

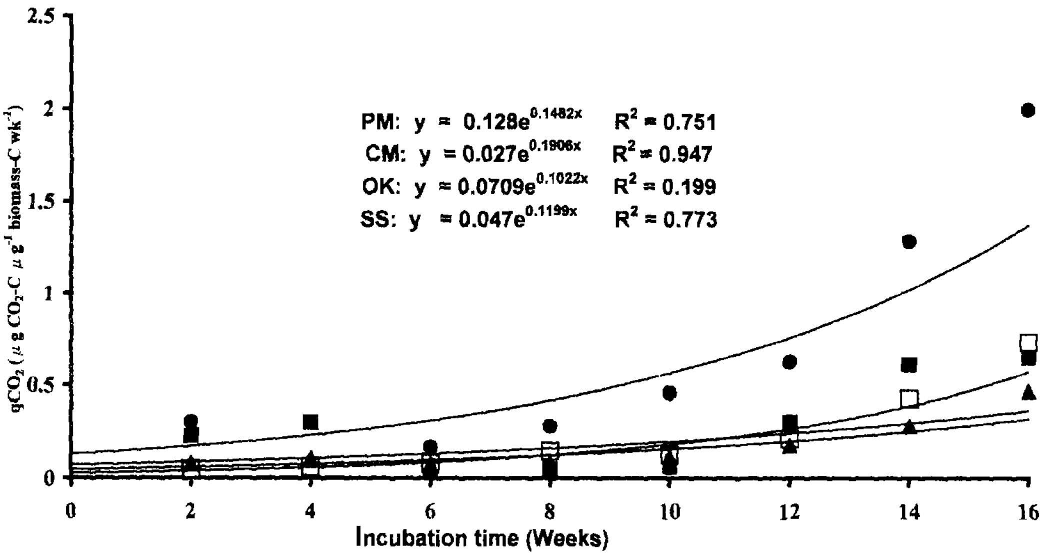

**Fig. 4.** Relationship between metabolic quotients ($qCO_2$) and incubation time: $qCO_2$(OK), $qCO_2$(CM), $qCO_2$(PM), $qCO_2$(SS) denote metabolic quotients for okara, cow manure, poultry manure, and sewage sludge composts, respectively. The lines represent the regression equations for the composts. ■ $qCO_2$(OK), □ $qCO_2$(CM), ● $qCO_2$(PM), ▲ $qCO_2$(SS).

which may account for the amount of biomass built up during this period.

The differences in the compost fractions probably reflected the differences in the growth and activity of the succession of microbial species, which are largely influenced by the concentration of readily available C in the substrates. As reported by Sakamoto and Hodono (2000), microbial biomass C shows a strong positive correlation with the turnover time of biomass C, since the turnover time is closely related to the survival of microorganisms. Kouno et al. (2002) also observed the increase in the total biomass C pool size and turnover time as a result of the addition of ryegrass to soil. The higher level of biomass C for OK shown in Fig. 3 at a 150 g kg$^{-1}$ application rate than in CM could be due to the activity of various microbial species, availability of easily decomposable substrates and their efficiency in decomposition. The observation of qualitative differences between microbial communities can be associated with differences in the microbial species. As for the selective adaptation of microbial populations to certain plant residues / composts, no conclusive evidence can be found in the literature (Verstraete and Voets 1977). The soil microbial biomass is thus involved in the decomposition of organic materials and cycling of nutrients in soil. Therefore, it appears that the mineralization potential of OK is slightly higher than that of the other composts, as evidenced by the lower C / N ratio (Table 2).

## Metabolic quotients ($qCO_2$)

The soil microbial biomass, as living microbial cells in soil, is the main agent responsible for $CO_2$ evolution. The ratio of $CO_2$ evolution to microbial biomass is designated as metabolic quotient: $qCO_2$ (Anderson and Domsch 1986). The $qCO_2$, or specific respiration rate, has been used in soil microbial biomass analyses for investigations on the maintenance of energy (Anderson and Domsch 1985a, b). The metabolic quotient is high when the microorganisms cannot fully metabolize C efficiently while feeding on N. Similarly, a higher $qCO_2$ also reflects the microbial response to adverse environmental conditions or disturbances (Wardle and Ghani 1995).

The metabolic efficiency of microbial biomass is an indication of the availability of organic C and its utilization. The $qCO_2$, therefore, evaluates the efficiency of soil microbial populations in utilizing organic C compounds. In this study, the $qCO_2$ decreased exponentially in the order of CM > SS > PM > OK, with ($p < 0.05$) and $R^2$ values of 0.95, 0.77, 0.75, 0.20, respectively (Fig. 4). A lower value was observed in the case of OK. Anderson and Domsch (1993) showed that a lower prevailing soil pH had a negative effect on $qCO_2$. This might explain the observation in OK, although in our data, a moderately high soil pH in CM, SS, and PM gave seemingly corresponding $qCO_2$ values. Comparatively, the metabolic quotient was higher in CM than in OK, implying that the microbes were under stress while utilizing CM and, therefore, could not fully metabolize C efficiently. This behaviour in microbial activities could be linked to the mineralization pattern of organic materials such as compost. The order of decomposition as indicated by the metabolic quotients was similar to that observed by the use of the C / N ratio in predicting rates. The observed $qCO_2$ was linked to the decomposability of the organic materials, as indicated in the $CO_2$-C evolution.

Therefore, the use of the metabolic quotient may also enable to predict the decomposability of organic materials such as compost, with the additional advantage of knowing the microbial actions involved in terms of biomass. The biomass is the most important indicator of microbial performance in soil, especially in combination with $CO_2$ production (Anderson and Domsch 1993). The diversity and composition of microbial populations should be taken into account to further strengthen $qCO_2$ actions, as indicated by the fact (Mamilov and Dilly 2002) that bacterial activities during decomposition built up microbial biomass. Soils with a heavy metal history should also be taken into consideration when calculating $qCO_2$ values, since heavy metal actions affect the metabolic activities of microorganisms (Insam et al. 1996).

While $qCO_2$ undoubtedly reflected the microbial efficiency for carbon utilization in this short-term study, Zaman et al. (2002) reported its inconsistency in long-term evaluation of organic wastes due to conflicting results in some studies. Although Zaman et al. (2002) did not discuss the cause of this phenomenon, this could be due to the fact that in a long-term evaluation, the microbial nutrient status changes considerably, depending on other environmental factors, the composition and the physiological state of the microflora, and the availability of decomposable substrates. However, $qCO_2$ could possibly be used as microbial indicator in the short term.

The soil microbial activity most probably depends on the biomass level and metabolic quotient. However, there was an inverse relationship between the biomass level in soil and the metabolic quotient (Santruckova and Straskraba 1991). This, therefore, suggests that, in some cases, higher levels of carbon inputs to the soil increase the microbial biomass but not the metabolic activity, which in turn leads to a decrease of the metabolic quotient (Insam et al. 1991).

Our results indicate that parameters such as microbial biomass and metabolic quotient are indices that affect the decomposability and carbon utilization of organic materials such as composts. The composts therefore provided additional C for microbial respiration and growth, but the fraction of decomposed C used for microbial efficiency might have been larger with a larger C supply, and $qCO_2$ plays a significant role in C efficiency, utilization, mineralization and in macroaggregate formation, as discussed by Miller and Dick (1995).

The addition of organic materials with easily degradable C compounds to soil appears to be suitable for stabilizing and supplying carbon and very useful from the viewpoint of C sequestration and ways of maintaining or increasing the humus content of soils. The order of $qCO_2$ seems to follow that of C and N mineralization.

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