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Kazunori Sakamoto · Yutaka Oba

Effect of fungal to bacterial biomass ratio on the relationship between CO_2 evolution and total soil microbial biomass

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Abstract The relationship between the fungal: bacterial biomass ratio and the metabolic quotient (qCO_2) was studied in three different soils. In addition, the effect of the fungal: bacterial biomass ratio on the relationship between CO_2 evolution and the size of the soil microbial biomass was examined. Soil samples were collected from three experimental fields amended with various organic materials (Yatsugatake, Ibaraki, and Tochigi fields). The range of the fungal: bacterial biomass ratio in the Yatsugatake and Ibaraki fields was small (1.54–2.24 and 1.11–1.71, respectively), but it was large in the Tochigi field (1.18–3.75). We found a high negative correlation between this ratio and the metabolic quotient ($qCO_2 = 2.10 - 0.361$ (fungal: bacterial biomass ratio), $R = -0.851$, $P < 0.01$) in the Tochigi field. Therefore, we suggest that qCO_2 decreases with an increase in the fungal: bacterial biomass ratio, which may be due to a higher efficiency of substrate C use by fungal flora in comparison with bacterial flora. In the Yatsugatake and Ibaraki fields, there was a high positive correlation between CO_2 evolution and total microbial biomass. In contrast, no correlation was observed between these two parameters in the Tochigi field, probably reflecting the wide range of values for the fungal: bacterial biomass ratio. From the results obtained, we suggest that the fungal: bacterial biomass ratio is an important factor regulating the relationship between CO_2 evolution and the size of the microbial biomass.

Key words CO_2 evolution · Microbial obiomass · Fungal: bacterial biomass ratio · qCO_2 · Organic manuring

K. Sakamoto (✉)
Faculty of Horticulture, University of Chiba,
Matsudo, 271 Japan

Y. Oba
Institute of Applied Biochemistry, University of Tsukuba,
Tsukuba, 305 Japan

Introduction

The CO_2 evolution has frequently been used to determine biological activity in soil in relation to changes in climate, in physical and chemical soil properties, and in agricultural practice (Nannipieri et al. 1990). In addition, the measurement has been used to obtain a better understanding of soil C turnover (mineralization and stabilization) and thereby to gain insight into how mineral nutrients and soil organic matter can be used more efficiently and conserved (Anderson 1982).

Soil microbial biomass, the living microbial cells in the soil, is the main agent responsible for CO_2 evolution. The ratio of CO_2 evolution to microbial biomass is termed the metabolic quotient, qCO_2 (Anderson and Domsch 1986). This quotient varies according to the composition and physiological state of the microflora, the availability of decomposable substrate, and various climatic conditions such as temperature and precipitation. Fungal flora, which is highly efficient in using substrate C, assimilates 30–40% of C into new mycelium (Alexander 1977; Yanagida 1984). In contrast, bacterial flora, which uses C inefficiently, assimilates 5–10% of substrate C into new cells (Alexander 1977; Yanagida 1984). Therefore, the fungal: bacterial biomass ratio may be an important factor affecting the value of qCO_2 since the fungal flora releases less CO_2-C for each unit of substrate C metabolized than the bacterial flora. No attempt, however, has been made to estimate the effect of the fungal: bacterial biomass ratio on qCO_2 and the relationship between CO_2 evolution and microbial biomass size.

In the present study we determined the size of the fungal and bacterial biomass, the fungal: bacterial biomass ratio, CO_2 evolution, and qCO_2 in agricultural soils amended with various organic materials, in an attempt to investigate the relationship between the fungal: bacterial biomass ratio and qCO_2 and the effect of this ratio on the relationship between CO_2 evolution and the size of the soil microbial biomass.

Materials and methods

Soil samples

The soils used have been described by Sakamoto and Oba (1991). Soil samples were collected from fields amended with organic material and located in three experiment stations: (1) Yamanashi Agricultural Experiment Station, Yatsugatake Substation, in Nagasaka (Yatsugatake); (2) Ibaraki Agricultural Experiment Station in Mito (Ibaraki); and (3) Tochigi Agricultural Experiment Station in Utsunomiya (Tochigi). Table 1 shows the treatments in the various plots and some properties of the soils in the fields surveyed. The field soils belonged to the following soil types: Yatsugatake, Ochric Andosols (FAO/UNESCO); Ibaraki and Tochigi, Humic Andosols. The control plot was the only plot amended with inorganic fertilizer while the other plots were amended with both organic material and inorganic fertilizer. The mean annual temperatures and precipitation in the surveyed fields are 13.9 °C and 1055.1 mm for Yatsugatake; 13.2 °C and 1307.8 mm for Ibaraki; and 13.0 °C and 1382.5 mm for Tochigi. According to plot size, four to six samples per plot were taken from the plowed layer (0–10 cm), bulked, and sieved (<5 mm). The water content of the sieved soil was adjusted to about 60% water-holding capacity. Then the soils were incubated under aerobic conditions for 10 days at 25 °C to allow the metabolic activities to become stabilized after sampling and sieving.

Fungal and bacterial biomass

The fungal and the bacterial biomass were measured by the direct microscopic method. The length and diameter of the fungal hyphae were measured by the membrane filter method (Hanssen et al. 1974), using optical microscopy and phenol aniline blue as a stain. Five membrane filters were prepared from each soil sample. A density of 1.1 g cm^{-3} and a dry weight of 20% were adopted for the fungal biomass calculations (Shields et al. 1973). The bacteria were enumerated by the nucleopore filter method (Hobbie et al. 1977), using fluorescence microscopy and acridine orange as a fluorescent stain. Three nucleopore filters were prepared from each soil sample. A bacterial volume of 0.19 μm^3 (Nishio 1983), a density of 1.1 g cm^{-3}, and a dry weight of 20% (Shields et al. 1973) were adopted for the bacterial biomass calculations.

ATP content

The ATP content in soil was determined by a slight modification of the method described by Jenkinson and Oades (1979). ATP was extracted with a solution containing trichloroacetic acid and phosphate but not paraquat (Inubushi et al. 1989) and determined with an ATP photometer (Aminco Chem Glow photometer J4-7441). The measurement was performed in triplicate.

CO_2 evolution

CO_2 evolution was determined with an infrared gas analyzer (Fuji Electric, ZAU1), using a dynamic method (Seto 1980; air flow rate, 0.5 liter min^{-1}) in the laboratory at 25 °C. The measurement was performed in triplicate.

Results

Microbial biomass and fungal : bacterial biomass ratio

In all the fields, the concentrations of the fungal, bacterial, and total microbial biomass were higher in the soils amended with organic material than in control soils amended only with inorganic fertilizer (Table 2). In soils amended with organic material the fungal : microbial bio-

Table 1 Treatment of plots with various organic materials and some characteristics of the soils in the fields surveyed

Plot	Organic material applied (t ha^{-1} $year^{-1}$)	Total C (%)	Total N (%)	pH (H_2O)
Yatsugatake field				
Control	0	3.79	0.335	5.46
Fallen leaves	3.7	4.52	0.367	5.72
Rice straw	4.6	4.59	0.390	5.57
Rice straw compost A	3.2	4.12	0.356	5.72
Rice straw compost B	6.4	4.50	0.384	5.96
Cattle feces	8.4	4.72	0.404	5.50
Ibaraki field				
Control	0	6.96	0.500	5.85
Wheat straw	14.0	7.63	0.542	5.95
Rice straw compost	4.4	7.54	0.536	5.72
Cattle compost	5.7	7.53	0.528	5.73
Sewage sludge compost	2.8	6.94	0.499	5.68
Swine feces	2.5	7.05	0.505	5.85
Tochigi field				
Vegetable-cropped series				
Control	0	9.41	0.626	5.23
Wheat straw + cattle compost	4.6 + 4.3	9.56	0.636	5.34
Corn straw + cattle compost	15.0 + 4.3	9.82	0.667	5.07
Cattle compost	8.6	9.68	0.661	5.39
Cereal-cropped series				
Control	0	9.38	0.611	5.51
Cattle feces	8.7	10.09	0.671	5.48
Swine feces	9.4	10.22	0.683	5.39
Cattle compost	8.6	10.01	0.698	5.96

Control, Plots were amended with inorganic fertilizer only. In the Tochigi field, the wheat straw and corn straw were applied for the summer crop, and the cattle compost for the winter crop

Table 2 Microbial biomass ($\mu g\ g^{-1}$ dry soil) in the fields surveyed

Plot	Fungal biomass	Bacterial biomass	Total biomass	Fm/Bm ratio
Yatsugatake field				
Control	201 ± 12	125 ± 18	326	1.61
Fallen leaves	258 ± 87	168 ± 32	426	1.54
Rice straw	300 ± 110	183 ± 32	486	1.64
Rice straw compost A	303 ± 82	135 ± 11	438	2.24
Rice straw compost B	346 ± 60	165 ± 11	511	2.10
Cattle feces	377 ± 80	194 ± 12	571	1.94
Ibaraki field				
Control	91 ± 27	82 ± 25	173	1.11
Wheat straw	244 ± 62	143 ± 6	387	1.71
Rice straw compost	176 ± 51	116 ± 19	292	1.52
Cattle compost	145 ± 39	118 ± 32	263	1.23
Sewage sludge compost	129 ± 44	99 ± 14	228	1.30
Swine feces	125 ± 25	103 ± 9	228	1.21
Tochigi field				
Vegetable-cropped series				
Control	200 ± 73	125 ± 13	325	1.60
Wheat straw + cattle compost	329 ± 104	133 ± 25	462	2.47
Corn straw + cattle compost	604 ± 87	161 ± 11	765	3.75
Cattle compost	221 ± 30	159 ± 36	380	1.39
Cereal-cropped series				
Control	198 ± 41	165 ± 13	363	1.20
Cattle feces	254 ± 81	259 ± 25	513	0.98
Swine feces	318 ± 72	249 ± 60	567	1.28
Cattle compost	310 ± 83	263 ± 9	573	1.18

Means ± SD. Fm/Bm ratio, fungal: bacterial biomass ratio

mass ratio was either high or low in comparison with that of the control, in each field (Table 2). The ratio was larger in the Tochigi field (1.18–3.75) than in the other fields (Yatsugatake field, 1.54–2.24; Ibaraki field, 1.11–1.71).

Table 3 ATP content and CO_2 evolution in the fields surveyed

Plot	ATP ($\mu g\ g^{-1}$ dry soil)	CO_2 evolution ($\mu g\ C\ h^{-1}\ g^{-1}$ dry soil)
Yatsugatake field		
Control	0.91 ± 0.06	0.389 ± 0.049
Fallen leaves	1.30 ± 0.04	0.590 ± 0.168
Rice straw	1.24 ± 0.11	0.514 ± 0.085
Rice straw compost A	1.14 ± 0.09	0.450 ± 0.049
Rice straw compost B	1.33 ± 0.27	0.624 ± 0.050
Cattle feces	1.56 ± 0.12	0.845 ± 0.085
Ibaraki field		
Control	0.90 ± 0.05	0.189 ± 0.063
Wheat straw	1.65 ± 0.05	0.825 ± 0.063
Rice straw compost	0.95 ± 0.03	0.356 ± 0.072
Cattle compost	1.16 ± 0.06	0.672 ± 0.159
Sewage sludge compost	0.85 ± 0.06	0.352 ± 0.036
Swine feces	0.81 ± 0.09	0.293 ± 0.036
Tochigi field		
Vegetable-cropped series		
Control	1.02 ± 0.20	0.408 ± 0.082
Wheat straw + cattle compost	1.50 ± 0.11	0.470 ± 0.048
Corn straw + cattle compost	1.88 ± 0.52	0.640 ± 0.097
Cattle compost	1.39 ± 0.10	0.724 ± 0.049
Cereal-cropped series		
Control	1.35 ± 0.24	0.628 ± 0.047
Cattle feces	2.06 ± 0.19	0.786 ± 0.048
Swine feces	1.78 ± 0.26	0.923 ± 0.085
Cattle compost	1.61 ± 0.12	1.079 ± 0.049

Means ± SD

ATP and CO_2 evolution

The ATP content and CO_2 evolution in the plots amended with organic material were also higher than those in the control plots, in all the fields (Table 3), except for ATP in the plots amended with sewage sludge compost and swine feces in the Ibaraki field.

Relationship between CO_2 evolution and total microbial biomass and between CO_2 evolution and ATP content

Figures 1–3 show the relationship between CO_2 evolution and total microbial biomass, and between CO_2 evolution and ATP content in the soil of each field. In the Yatsugatake and Ibaraki fields, there was a highly positive correlation between CO_2 evolution and total microbial biomass, and between CO_2 evolution and ATP content ($R = 0.849-0.956$). However, there was no correlation between these parameters in the Tochigi field.

Relationship between total microbial biomass and qCO_2, and between the fungal: bacterial biomass ratio and qCO_2

Figure 4 shows the relationship between total microbial biomass and qCO_2 in the soil of the surveyed fields. There was no correlation between total microbial biomass and qCO_2 in any of the fields. Figure 5 shows the relationship between the fungal: bacterial biomass ratio and qCO_2. In the Yatsugatake and Ibaraki fields, there was

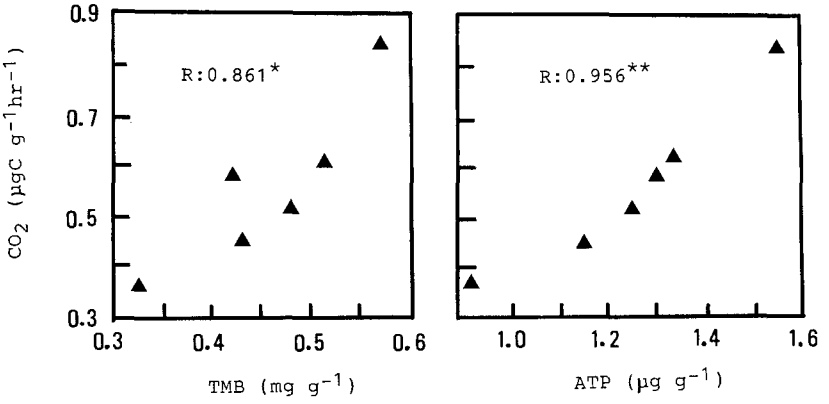

Fig. 1 Relationship between CO_2 evolution and total microbial biomass (*TMB*), and between CO_2 evolution and ATP content in the Yatsugatake field

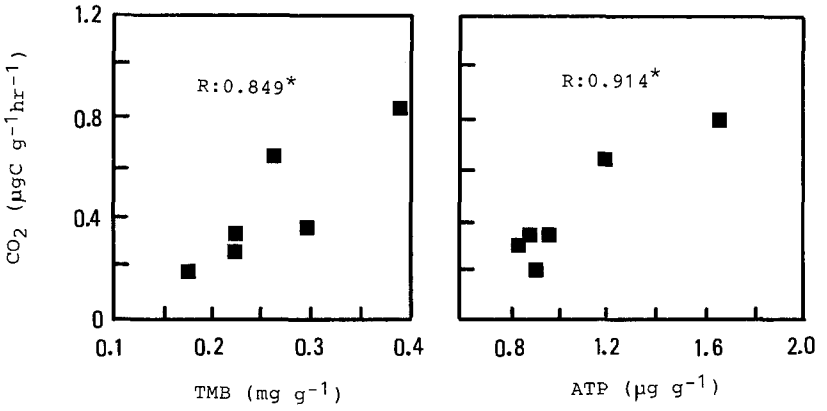

Fig. 2 Relationship between CO_2 evolution and total microbial biomass (*TMB*) and between CO_2 evolution and ATP content in the Ibaraki field

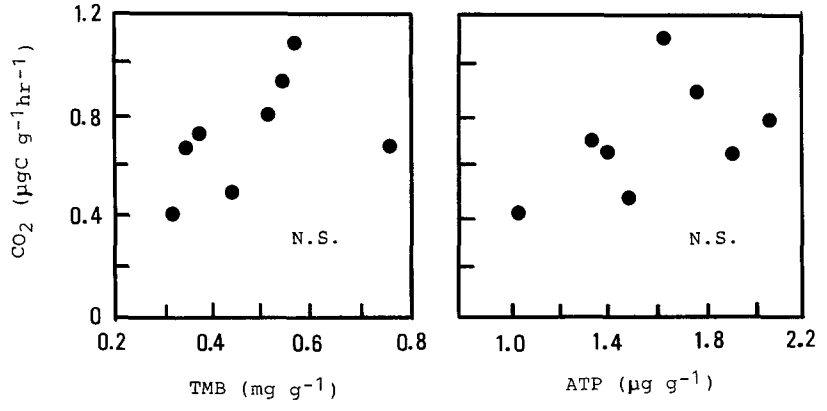

Fig. 3 Relationship between CO_2 evolution and total microbial biomass (*TMB*) and between CO_2 evolution and ATP content in the Tochigi field

no correlation between this ratio and qCO_2. However, there was a highly negative correlation between the two parameters in the Tochigi field ($Y = 2.10 - 0.361X$, $R = -0.851$, $P < 0.01$).

Discussion

We found a highly negative correlation between the fungal: bacterial biomass ratio and qCO_2 in the Tochigi field, perhaps reflecting greater efficiency in the use of substrate C by the fungal flora in comparison with the bacterial flora. In the Yatsugatake and Ibaraki fields, there was no correlation between the fungal: bacterial biomass ratio and qCO_2. This may have been due to the small range of values for the fungal: bacterial biomass ratio in the soils of these fields, while a large range of values was found in the soils of Tochigi field. This large range may also explain our finding that the CO_2 evolution was not correlated with the total microbial biomass and ATP content of the soil. Indeed, the large range in values for the fungal: microbial biomass ratio may have been responsible for the diverse range of qCO_2 values. From the results obtained, we suggest that the fungal: microbial biomass ratio is an important factor regulating the relationship between CO_2 evolution and the size of the microbial biomass.

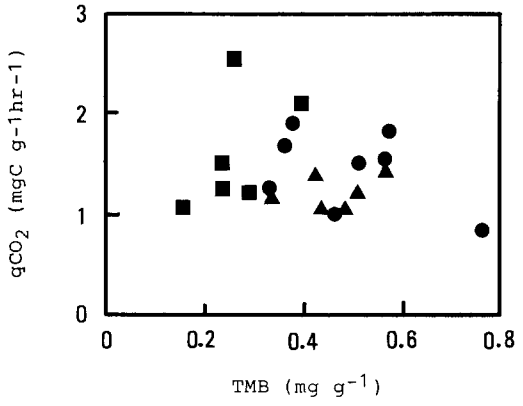

Fig. 4 Relationship between total microbial biomass (*TMB*) and the metabolic quotient, qCO_2. ▲, Yatsugatake field (NS); ■, Ibaraki field (NS); ●, Tochigi field (NS)

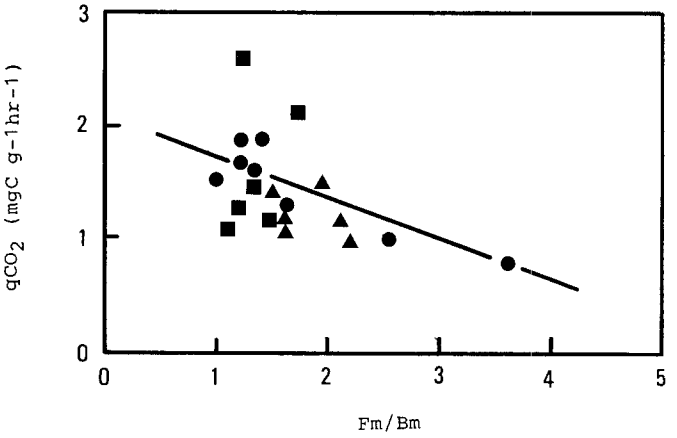

Fig. 5 Relationship between fungal:bacterial biomass ratio (*Fm/Bm*) and the metabolic quotient, qCO_2. ▲, Yatsugatake field (NS); ■, Ibaraki field (NS); ●, Tochigi field ($qCO_2 = 2.10 - 0.361$ (Fm/Bm), $R = -0.851$**). The *line* represents the regression equation for the Tochigi field

In order to examine the validity of our hypothesis, we recalculated the coefficient of regression between CO_2 evolution and microbial biomass for the Tochigi field (Fig. 6), except for plots in which the fungal:microbial biomass ratio was either high or low in comparison with values in the Yatsugatake and Ibaraki fields (1.11–2.24). The excluded plots were wheat straw+cattle compost (fungal:microbial biomass ratio 2.47), corn straw+cattle compost (3.75), and cattle feces (0.98). As shown in Fig. 6, there was a high positive correlation between CO_2 evolution and total microbial biomass and also ATP content by this method. This result supports the validity of our hypothesis.

The relationship between CO_2 evolution and microbial biomass size has been examined in field soils. Some workers have found a clear positive correlation between CO_2 evolution and microbial biomass (Oades and Jenkinson 1979; Ross et al. 1980; Sparling 1981; Hasebe et al. 1985), but other workers have not (Kaczmarek et al. 1976; Frankenberger and Dick 1983). Therefore, the functional relationship between CO_2 evolution and microbial biomass is not yet fully understood. Recently, the ratio of CO_2 evolution to microbial biomass (qCO_2) was related to Odum's (1969) theory in terms of energetic optimization during ecosystem development (Insam and Domsch 1988; Insam and Haselwandter 1989). The qCO_2 value was high in "young" soils at the early stage of ecosystem succession (fallow and field) and low in "mature" ones at a stage (meadow and forest). This suggests that competition for the available C source favors microorganisms (or communities) needing the least amount of energy for maintenance and growth and thus releasing less CO_2. However, changes in the fungal and bacterial biomass during ecosystem succession have not yet been determined. The fungal flora seems to be a suitable parameter to use in comparison with the bacterial flora when studying advanced stages of ecosystem succession because the activity of hydrolytic enzymes in fungal flora, such as cellulose, is generally greater than that of the bacterial flora (Alexander 1977; Campbell 1983). It seems important, therefore, to examine the changes in fungal and bacterial biomass during an ecosystem succession.

Šantrůčková and Straškraba (1991), who investigated CO_2 evolution and the microbial biomass in soils at various stages of a secondary succession, reported a steep negative hyperbolic relationship between qCO_2 and mi-

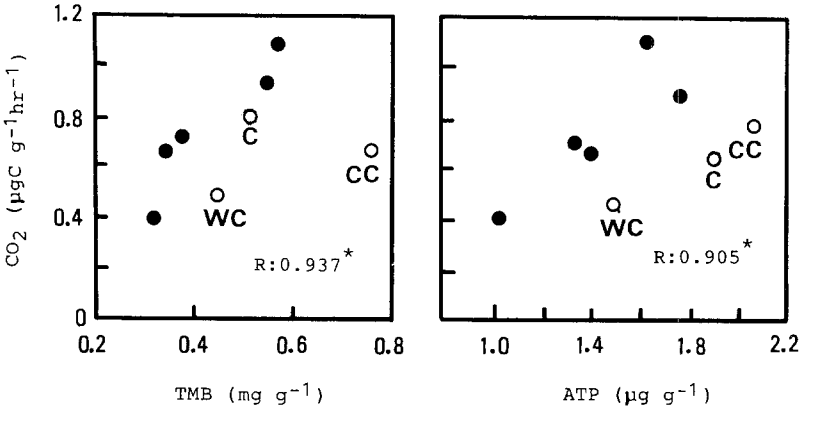

Fig. 6 Recalculated relationship between CO_2 evolution and total microbial biomass (*TMB*), and between CO_2 evolution and ATP content in the Tochigi field, omitting plots in which the fungal:bacterial biomass ratio differed from the range found in the Yatsugatake and Ibaraki fields. Treatment of omitted plots (○): *WC*, wheat straw+cattle compost; *CC*, corn straw+cattle compost; *C*, cattle feces

crobial biomass. They concluded that microbial respiration was inhibited by high concentrations of CO_2, which increased the amount of microbial biomass produced. The results of the current study, however, do not support their hypothesis because there was no correlation between qCO_2 and microbial biomass in any of the surveyed fields (Fig. 4). The reason for the different results obtained by Šantrůčková and Straškraba compared with the current study is unclear. Further studies are needed to resolve the question.

We conclude that the fungal: bacterial biomass ratio is an important factor regulating the relationship between CO_2 evolution and the size of the microbial biomass. However, further investigations in various types of soil ecosystem should be carried out in order to examine the validity of our hypothesis.

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