

Size and Activity of the Soil Microbial Biomass and Soil Enzyme Activity in Long-Term Field Experiments

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Abstract: Soil microbial biomass carbon, microbial respiration and soil enzyme activity were estimated from six different manure treatments: a) effective microorganism (EM) compost 15 t ha⁻¹ (EM1), b) traditional compost 15 t ha⁻¹ (TC1), c) EM compost 7.5 t ha⁻¹ (EM2), d) traditional compost 7.5 t ha⁻¹ (TC2), e) chemical fertilizer (CF), f) control (no any manure, CK). The experimental results showed that soil microbial biomass C, microbial respiration rate and enzyme activity in compost system were significantly higher than in CF system and CK system. Alkaline phosphatase and urease activity in CF system were significantly higher than in CK system. The microbial biomass C and microbial respiration rate in treatments followed the order: CK<CF<TC<EM. Phosphatase and urease activity in treatments followed the order: control<chemical fertilizer<compost. Therefore, size and activity of soil microbial biomass and soil enzyme activity were increased due to applied compost. The metabolic quotient in treatments followed the order: EM1<TC1<EM2<TC2<CF<CK. The highest value of the metabolic quotient was found in CK system, which reflected a stress in low supply of substrate carbon. Correlation analysis revealed highly significant relationships for all combinations microbial biomass C, microbial respiration rate and phosphatase and urease activity. Significant correlations also were observed among soil chemical properties (organic matter and N, P, K) and between soil nutrients and microbial biomass C, microbial respiration rate and phosphatase and urease activity. Therefore, size and activity of microbial biomass, microbial metabolic quotient and soil enzymes activity could reflect condition of soil fertility and should be considered as important bio-indicators of changes in soil quality.

Key words: Metabolic quotient • manure • microbial respiration • soil enzyme, • soil microbial biomass

INTRODUCTION

Long-term benefits from applied organic manure include bettered tilth, improved water-holding capacity and cation exchange capacity, moderated soil temperature and enhanced crop performance, increased soil organic matter and increased biological activity [1, 2], moreover, it can suppress soil-borne plant diseases [3, 4]. So soil fertility and crop yield was increased and good soil physical or chemical property and biological community was kept, furthermore there had a healthy and sustainable soil environmental condition. Effective Microorganism (EM) compost had both traditional compost and additional effective microorganism benefit. Moreover, application of chemical fertilizer and pesticides was lessened due to applied EM compost.

Soil microorganisms are main participant in soil formulation and nutrient cycling [5]. Size and activity of soil microbial biomass are greatly stimulated by the addition of manure. Soil microbial biomass is the living component of soil organic matter [6, 7]; and it generally comprises 1-5% of total organic matter content [8]. Because of its high turnover rate, microbial biomass C could respond more rapidly to changes of soil environment than soil organic matter [9]. Measurement of the size of soil microbial biomass couldn't indicate microbial activity. Microbial activities include basal respiration rate and the activities of general enzymes such as alkaline phosphatase and urease. Phosphatases are involved in the transformation of organic and inorganic phosphorus compounds in soil [10]; and ureases are involved in releasing inorganic N in the N cycle [11].

Moreover, microbial biomass and enzyme activities have been shown to be more sensitive than total carbon concentration to soil management practices [12, 13]. Microorganisms were responsible for the decomposition and mineralization of the organic matter. Soil respiration, metabolic quotient and soil enzymatic activity are adequate indicators of microbial activity and modifications occurred in the soil due to the addition of organic and inorganic manure [14]. Therefore, soil microbial biomass, microbial respiration, the metabolic quotient (qCO_2) and soil enzymatic activity can be utilized as indicator for changes in soil quality produced by agricultural management practices [5].

The purpose of this study was to investigate the effects of long-term farming management practices on size and activity of soil microbial community and activities of soil enzyme, as well as the correlation between them and soil other physico-chemical properties.

MATERIALS AND METHODS

Experimental site and design: A long-term field experiment in winter-wheat and summer-corn farming was initiated in 1993 at Qu-Zhou experimental station ($36^{\circ} 52'N$, $115^{\circ} 01'E$), China Agricultural University, in Qu-Zhou county, Hebei province, Northern China. The station is in a continental temperate monsoon zone and the climate in the region is warm, sub-humid and consists of summer rainfall and dry-cold winters. The mean annual temperature is $13.2^{\circ}C$ and ranges from a minimum of $-2.9^{\circ}C$ in January to a maximum of $26.8^{\circ}C$ in July, mean annual precipitation is 542.7 mm, of which 60% occurs from July to September and the annual non-frost period is 201 d. The mean annual evaporation is 1841 mm and is more three times annual rainfall, so spring drought is very severe. Light, heat and water resources are abundant and shallow surface groundwater has high mineral component and groundwater level is high. The soil at study site is an improved silt fluvo-aquic soil.

Each of six combinations was repeated three times for a total of eighteen plots, laid out in a randomized complete block design. Plots, 3×10.5 m each, were planted with winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) every year from 1993 to 2004. The experiment was designed with six treatments: 1) applied EM compost 15 t ha^{-1} (EM1), 2) applied traditional compost 15 t ha^{-1} (TC1), 3) applied EM compost 7.5 t ha^{-1} (EM2), 4) applied traditional compost 7.5 t ha^{-1} (TC2), 5) applied chemical fertilizer (CF) (750 kg ha^{-1} ammonium bicarbonate, 300 kg ha^{-1} urea and 750 kg ha^{-1} calcium super-phosphate), 6) control (no any manure, CK). Before planting winter wheat and summer maize every year, EM1 and EM2 plots were treated with EM

compost and TC1 and TC2 plots with traditional compost, at the same time CF plot was applied with ammonium bicarbonate, urea and calcium super-phosphate, respectively, while CK plot didn't receive any manure. Every 50 kg traditional composts were composed of 30 kg straw (wheat straw in June or maize straw in October), 15 kg livestock dung, 5 kg cottonseed-pressed trash and in addition 1 kg red sugar. Every 50 kg EM composts (Effective microorganism agent was made in Beijing Yiaimu biotechnology company.) were that traditional composts appended with 200 ml solutions of effective microorganism agent. Effective microorganism consisted of more 80 kinds of microbes were mixed and incubated, including photosynthesis microbes, acetate bacillus, actinomycetes, lactobacillus, microzyme, etc.

Soil sampling and physico-chemical analysis: Soil samples were collected in June of 2004 from depths of 0-20 cm at 15 soil cores (3 cm diameter) in each plot, stored in an insulated plastic bags tied and prevented moisture loss and as soon as possible transferred to a $4^{\circ}C$. The soil samples were mixed gently prior to removal of aliquots for assays. Aliquots of samples were sieved through a 2-mm screen at field-moist conditions and mixed to determine soil moisture and to analyze microbial properties. Soil moisture in each sample was determined by weight loss after heating at $105^{\circ}C$ for 24 h and expressed as a percent dry weight. Samples were then air-dried for 14 d at room temperature, sieved through a 1-mm screen, mixed and sub-sample were arrayed for soil enzyme, alkali-hydrolysable N, available P, available K and soil pH values. The other sub-samples were ground to pass through a 0.25-mm sieve to determine organic matter content and total N. The potassium dichromate external heating method [15], the semi-micro Kjeldahl method [16], the alkaline hydrolysable diffusion method [17], the classical Olsen method [15] and the ammonium acetate flame photometry method [18] were applied to determine organic matter, total N, alkaline hydrolysable N, available P, available K. Soil pH was measured in 0.01 mol l^{-1} $CaCl_2$ slurry (soil : solution = 1 : 2.5) using a glass electrode.

Soil microbial biomass, respiration and enzyme activity array: Soil microbial biomass C (MBC) was measured by a fumigation and extraction procedure described by Vance *et al.* [19]. Briefly, one portion of unfumigated soil (20.0 g field moisture soil) was extracted with 80 ml of 0.5 mol l^{-1} K_2SO_4 by shaking for 30 min and the suspension filtered using a Whatman No. 2 filter paper. A separate portion was fumigated by exposing soil to alcohol-free $CHCl_3$ vapor for 24 h in a vacuum desiccator

[20]. After CHCl_3 was removed by vacuum extraction, the fumigated portion was extracted with K_2SO_4 as above. Organic C in the extracts was analyzed using digestion-titration method [21]. Namely, After transferring 10 ml of extract to a test tube, 5 ml 33 mmol l^{-1} $\text{K}_2\text{Cr}_2\text{O}_7$ and 5 ml of conc. H_2SO_4 were added. The samples were digested for 10 min at 175°C and titrated using 0.05 mol l^{-1} FeSO_4 with 1,10-phenanthroline ferrous sulfate as the indicator. The amount of soil MBC calculated using the method: the difference between fumigated and non-fumigated samples divided by the K_2SO_4 extract efficiency factor for microbial C ($\text{Kc} = 0.379$). The microbial quotient was calculated by microbial biomass C as a percentage of total organic carbon [22, 23].

Microbial respiration was determined by placing 50 g field moisture soil into 50 ml beakers and incubating the samples in the dark at 25°C in 1000 ml, sealed jars along with a beaker contained 5 ml of 1 mol l^{-1} NaOH solutions, which captured respired CO_2 [24]. Then, the NaOH solution was removed and titrated to determine the amount of CO_2 evolved with the soil microbial respiration. The metabolic quotient ($q\text{CO}_2$) was obtained by dividing the basal respiration by the microbial biomass C. The results of microbial biomass C and microbial respiration were calculated using oven-dried soil (105°C , 24 h).

The soil alkaline phosphatase and urease activity were based on the release and quantitative determination of the product in a reaction mixture, the soil sample being incubated with suitable buffer solution. Assays were performed to determine the activity of enzymes as described by Tabatabai [25].

Statistical analysis: One-way variance analysis (ANOVA) was used to detect the effects different manure measurement on MBC, microbial respiration, enzyme activity and soil physico-chemical properties. Difference at $p < 0.05$ level was considered as statistically significant using the LSD (least significant difference) test. Bivariate correlations (Pearson, two-tailed) were used to explore

the relationships of MBC, microbial respiration, enzyme activity and soil chemical properties. All statistical analyses were performed by SPSS 11.5 software package.

RESULTS

Soil microbial biomass C and microbial respiration:

The highest microbial biomass C and microbial respiration rate were found in EM1 plot and the lowest were found in CK plot (Fig. 1). The microbial biomass C and microbial respiration rate in treatments had ascending trend as following: $\text{CK} < \text{CF} < \text{TC} < \text{EM}$. The result showed that farming management practice had extremely significant effect on microbial biomass C and microbial respiration ($p < 0.01$).

Microbial biomass C in compost system was significantly higher than CF and CK system ($p < 0.05$). Microbial biomass C in compost system was higher from 30.08 to 56.67% than in CF system and higher from 53.79 to 88.76% than in CK system. The contents of microbial biomass C in the treatments applied with different amount of compost had significant difference ($p < 0.05$), but there hadn't significant difference among the treatments applied the same amounts of compost. Microbial biomass C in CF plot was higher 18.23% than in CK, but no significant difference was observed.

The microbial respiration rates in compost system were significantly higher than CF and CK system ($p < 0.05$). The microbial respiration rates in compost system were higher from 21.62 to 26.67% than CF system and higher from 41.35 to 47.22% than CK system. There hadn't significant difference among compost system. Microbial respiration rate in CF plot was higher 16.22% than CK plot, but significant difference wasn't found between CF and CK plot.

The soil microbial quotient was high in compost systems and soil microbial quotient in compost systems was significantly higher than in CK ($p < 0.05$). The values ranged from 1.65 to 1.88% and the lowest value was

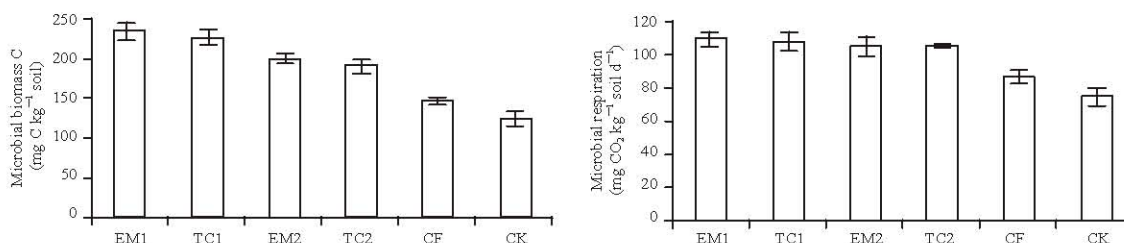


Fig. 1: Soil microbial biomass C and microbial respiration in different farming management system

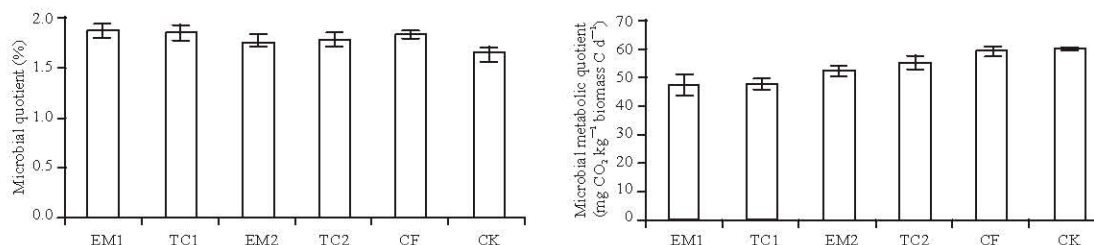


Fig. 2: Soil microbial quotient and metabolic quotient in different farming management system

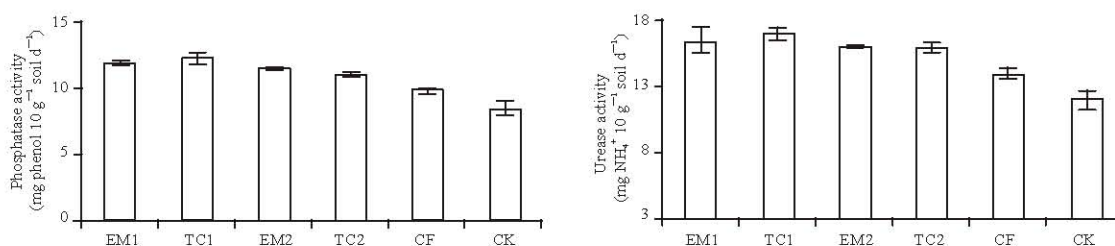


Fig. 3: Soil alkaline phosphatase and urease activity in different farming management system

in CK and the highest value was in EM1. No significant difference was revealed between compost and CF system.

The soil microbial metabolic quotient was high in CF and CK plot and soil microbial metabolic quotient in CF and CK plot was significantly higher than in EM1 and TC1 plot ($p < 0.05$). The metabolic quotient in treatments followed the order: EM1 < TC1 < EM2 < TC2 < CF < CK (Fig. 2). Furthermore, the microbial metabolic quotient in compost system hadn't significantly different except TC2. The metabolic quotient had no significant difference between CF and CK treatments.

Soil enzyme activities: Soil alkaline phosphatase and urease activity showed similar trends in response to different farming management practice (Fig. 3). The highest phosphatase and urease activity were found in TC1 plot and the lowest were found in CK plot. Values of phosphatase and urease activity in treatments generally followed the order: control < chemical fertilizer < compost. The alkaline phosphatase and urease activity had significant differences among treatments ($p < 0.01$).

With the increasing the amount of compost application, the alkaline phosphatase activity was improved. Phosphatase activity in compost system was significantly higher than CF and CK system ($p < 0.05$). Phosphatase activity in compost system was higher from 13.27 to 25.62% than CF system and higher from 30.92 to 45.18% than CK system. Phosphatase activity in systems applied different amount compost also had

significant difference ($p < 0.05$), but there hadn't significant difference between the same amounts of compost system. Phosphatase activity between CF and CK plot had also significant difference ($p < 0.05$). Phosphatase activity in CF plot was higher 15.58% than CK plot.

Urease activity in compost system was significantly higher than CF and CK system. Urease activity in compost system was higher from 14.45 to 21.85% than CF system and from higher 32.45 to 41.01% than CK system ($p < 0.05$). Urease activity in CF plot was higher 15.72% than CK plot and had significant difference ($p < 0.05$), but there hadn't significant difference among compost system.

Soil organic matter, N, P and pH: One-way variance analysis showed that the contents of soil organic matter (OM), total N, alkaline hydrolysable N, available P, available K and soil pH value had significantly different ($p < 0.01$). All the soil nutrient contents were higher in EM1 and TC1 than in EM2 and TC2 and were higher in EM2 and TC2 than in CF and CK. Except available K, soil nutrient contents were greater in CF than in CK, but the difference wasn't significant except available P. The soil nutrient contents were larger in EM1 than in TC1, but the difference wasn't significant except available P. The soil nutrient contents except alkaline hydrolysable N were larger in EM2 than in TC2 plot, but the difference wasn't significant (Fig. 4).

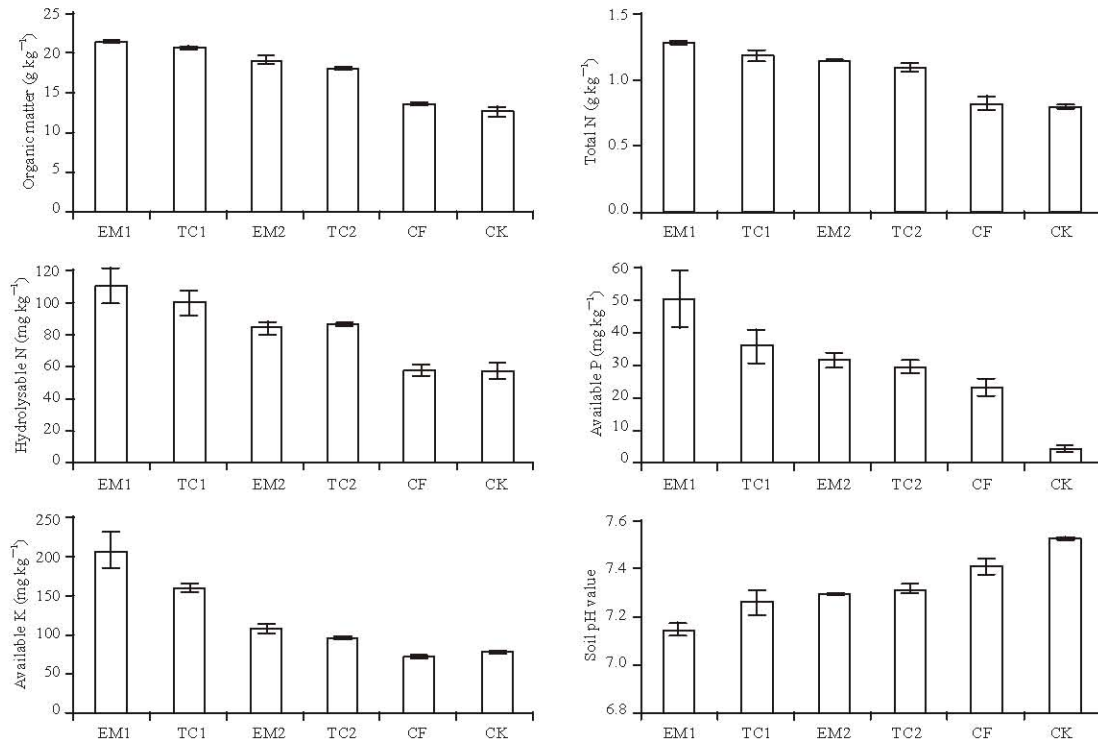


Fig. 4: Soil physicochemical properties in different farming management system

Table 1: Linear correlation coefficients (r) between soil microbial biomass carbon, microbial respiration, soil enzyme and soil physicochemical properties

Variable	A	B	C	D	E	F	G	H	I	J
MBC (A)	1.000	0.875**	0.904**	0.838**	0.967**	0.932**	0.883**	0.838**	0.770**	-0.843**
MR (B)		1.000	0.846**	0.851**	0.885**	0.841**	0.781**	0.741**	0.576*	-0.848**
Phosphatase (C)			1.000	0.919**	0.923**	0.895**	0.847**	0.824**	0.702**	-0.823**
Urease (D)				1.000	0.881**	0.844**	0.881**	0.863**	0.704**	-0.828**
Organic matter (E)					1.000	0.970**	0.911**	0.815**	0.797**	-0.872**
Total N (F)						1.000	0.880**	0.797**	0.807**	-0.850**
Hydrolysable N (G)							1.000	0.880**	0.875**	-0.829**
Available P (H)								1.000	0.814**	-0.875**
Available K (I)									1.000	-0.787**
pH value (J)										1.000

*, ** Significant at 0.05 and 0.01 levels of probability, respectively, 2-tailed, n = 18. MBC: Microbial Biomass Carbon; MR: Microbial Respiration

Correlation between microbial biomass, microbial activity, enzyme activity and soil chemical properties:

Correlation analysis revealed highly significant relationships for all combinations microbial biomass C concentrations, microbial respiration rates, phosphatase and urease activity ($p < 0.01$, Table 1). High significant correlations also were observed among soil nutrients (OM and N, P, K) ($p < 0.01$). Soil nutrients were significantly correlated with concentrations of microbial biomass C, microbial respiration rates, phosphatase and urease activity ($p < 0.05$). Soil pH value was negatively correlated with soil biological properties and soil nutrients ($p < 0.01$).

DISCUSSION

Microbial biomass C, basal respiration and enzyme activity in different farming management system: Soil microbial biomass C, microbial respiration and enzyme activity affected by farming management practice were observed. The microbial biomass C, microbial respiration rate and enzyme activity in compost system were significantly higher than in CF and CK system, as was consistent with previous researching result [26-28]. The microbial biomass C contents and phosphatase activity would heighten with the amount of applied compost increased and there had significant difference among

compost system ($p < 0.05$). The most important factor which differentiated the microbial communities and activities in the different farming management systems is the amount of C inputting the systems because C was often a limiting factor for soil microbial communities and activities [27]. Microbial respiration rates and urease activity hadn't significant difference among compost system, but microbial biomass C had significant difference ($p < 0.05$). The possible reason was that microbial number had a difference, but microbial activity hadn't significant difference among compost system. Microbial biomass C generally accounts for 1-5% of total organic carbon content. In the present study, this value ranged from 1.65 to 1.88% and the lowest value was in CK system and the highest value was in EM1 system and these values are within the reported range. So, the living component of soil organic carbon was high due to compost input [6, 29]. The ratio of MBC to total organic carbon could differentiate with applied compost and no manure input systems, but couldn't differentiate with compost and chemical fertilizer systems.

Significant difference was observed in alkaline phosphatase activity among different compost treatments, yet urease activity hadn't significant difference. This manifested that phosphatases were more sensitive to compost input than urease.

Phosphatase and urease activity were significantly higher in CF system than in CK system ($p < 0.05$). Though there hadn't significant difference for microbial biomass C and microbial respiration rates between CF system and CK, microbial biomass C and microbial respiration rates were higher in CF system than in CK. The reason was that high levels of chemical fertilizers increased root biomass and crop residue and improved soil organic carbon, so the size and activity of microbial community were improved [30].

Correlation between soil biological character and physicochemical properties: Soil microbial biomass C, microbial respiration rate and enzyme activity were significantly correlated with soil organic matter, total N, alkaline hydrolysable N, available P, available K concentration ($p < 0.05$, Table 1) [31]. This revealed that soil biological communities played crucial role in soil fertility formation and nutrient cycling and they could not only provide plant-available nutrients, but also accumulate soil organic carbon [32].

The evidence that the indices of microbial activity (microbial respiration rate, phosphatase and urease activity involved in N and P cycling) were closed

correlated with each other and with both organic matter and microbial biomass C content, indicates that substrate availability was the main factor influencing the size and activity of microbial community [8, 22]. Moreover, as well as being a substrate for microbial activity, soil organic matter played an important role in protecting soil enzymes since they could be immobilized in a three-dimensional net-work of clay and humus complexes [8].

The concentrations of soil organic matter, total N, available P, available K, microbial biomass C and microbial respiration rate after addition of EM agent all were increased. The main fact was that effective microorganism agent were consisted of more 80 kinds of microbes and stimulated organic materials decomposed and plant-available nutrients released.

Soil quality bioindicators: The experimental result indicated that microbial biomass C content, microbial respiration rates and enzyme activity all had significant response to different farming management and hence appeared to be useful for monitoring changes of soil quality [14, 33-36]. But biological index had different sensitivity to soil disturbance [38, 38]. Microbial biomass C, microbial respiration rate and enzyme activity all could differentiate between compost system and no compost system, so they were sensitive to compost input. But phosphatase and urease activity had significant difference between in CF and in CK system ($p < 0.05$), so phosphatase and urease were sensitive to chemical fertilizer input. The biological index responded differently to diverse amount of compost and microbial biomass C and phosphatase activity had significant difference among compost treatments. Nevertheless, no significant differences were observed in microbial respiration rates and urease activity between different compost treatments. Therefore, it would be difficult to establish a single biological or chemical criterion that could adequately reflect soil quality because of the multitude of microbiological components and biochemical pathways [11, 39]. However, microbial biomass C, microbial respiration rate and soil enzymes activity should be considered as one of important bioindicator [5].

Microbial metabolic quotient was mineralised per unit of microbial biomass carbon and per unit of time. Microbial metabolic quotient often considered as an indicator of microbial stress and soil quality [7, 40]. The values for metabolic quotient in CF system and in CK than in compost systems and there had significant

difference between in CF and in CK system and in EMI and TC1 system ($p < 0.05$). Microbial communities in those plots likely lived under starvation stress (i.e., low supply of substrate C), as was showed from soil organic matter content [28]. In other words, unfavourable conditions resulted in decline in the size of the microbial biomass and in the efficiency with which it used C substrates. Accordingly, there was an enhancement in respiration rate per unit of microbial biomass [41].

In conclusion, size and activity of microbial biomass, microbial metabolic quotient and soil enzymes activity reflected condition of soil fertility and should be considered as important indicators of changes in soil quality.

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