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Microbial biomass and microcalorimetric methods in tropical soils

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Abstract

The type of organic matter (OM) plays an essential role in nutrient cycling in agricultural soil systems. Microbial activity in tropical soils was calorimetrically followed as a useful tool in this investigation. Tropical soil samples with different textures: Rhodic eutrudox (R), Typic eutrudox (V) and a Quartzipsamment (Q) from Brazil were amended with 25% cattle manure (E), municipal refuse compost (L), earthworm casts (H), the agrochemical trifluralin (T); (23 μg, equivalent dose of 1.25 kg ha^{-1}) were explored. The microbial activity was determined by calorimetry and simultaneously by fumigation–extraction (microbial biomass carbon, C) to compare both methods. The results for R, Q, and V soils were: (212.04^{A}, 195.99^{B}, 204.47^{A}) for microbial biomass C and (0.692^{B}, 0.714^{B}, 0.784^{A}) for thermal effect with $P < 0.05$, respectively, over a period of incubation of 91 days. The microbial activity of the modified soils decreases in the order: E, H, L and T. Both methods showed a coefficient of correlation $r = 0.7443$ and the statistical probability of occurrence of the event, $P < 0.0001$. From this correlation the utility of both methods for measuring the microbial activity in soils could be deduced.

Keywords: Microbial activity; Biomass; Tropical soil; Microcalorimetry

1. Introduction

Organic matter (OM), microorganisms and plants are some components of the environment that continually influence agricultural systems. The diversity of conditions in the Tropics makes the OM a vitally important source of essential nutrients in tropical region. The specific biological processes involved in transformations of soil OM and their dynamics can contribute to agricultural systems in such regions. The labile constituents of OM are decomposed in a few weeks or months, while their stable components may persist in soil for years or decades. Soil microorganisms degrade a major part of labile fractions. The amount of materials degraded in the soil can be estimated by soil microbial biomass. The active pool of OM plays an essential role in short-term nutrient turnover in soil. Although there are still problems associated with the measurement of soil microbial biomass, the amount of carbon (C) contained in the biomass and stored in the total soil organic C ranged from 1 to 3%. Then the importance in measuring microbial biomass is related to turnover in total soil OM [1].

The materials available for microbial degradation in soil can vary in amount and chemical composition in different managed ecosystems. All of them differ in chemical composition, the quantities available in various ecosystems and degradation rates [2]. Death and life are continuously changed, and each state

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is dependent upon the other. The essential elements required by living cells include carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur and other elements, whose availability can be determined by reactions in the soil [3]. The diversity of minerals found in many tropical soils, and the OM present can strongly affect the soil characteristics and its ability to retain compounds and ions in its natural structure. Soil fertility depends on the bioreactions that take place by involving the microorganisms in the soil, which cause direct consequences on the development of plants and other organism [3].

The classic methods used for soil analysis are of great importance for measuring the activity of the microbial community. Soil microorganisms and the process that they govern are essential for long-term sustainability of agricultural systems [4]. The microbial biomass is related with OM in soil that plays essential roles in decomposition, nutrient cycling and biophysical manipulation of the native soil structure. One approach to study the dynamics of microbial ecosystems is related to model systems and determines the behavior of any model under defined condition [2].

Soil microbial activity is assessed through traditional methods, including carbon dioxide evolution [5] and enzymatic activities [6]. Microbial biomass C estimates the potential of the soil microbial activity [7,8], however, each method has its variation and limitations [9,10]. Comparative growth measurements provide an interesting challenge in several areas of agriculture and also important information in comparative studies of soil microbial activity. However, such diversity in activity can be monitored by calorimetry. This technique detects the thermal effect evolved in a given system, with an instrument, which is configured to monitor the thermodynamics of the biological process. Thus, the calorimetric method has been proven very suitable for measuring the soil microbial activity [11,12].

The thermal effect resulted from each microorganism metabolism is an important value for studying characteristics of microbial activity. In such method, all aerobic and anaerobic events recorded are quantified through the enthalpy values. In this way, calorimetry appears as a suitable technique to follow the biological process in heterogeneous systems. In recent years, significant progress has been made in the use of the microcalorimetric method as a microbiological technique [13] and also involving the glucose degradation to provide information on the soil microbial activity [14].

The aim of this investigation is to compare in a same system, the thermal effect assessed through microcalorimetry with a classical microbiological method, the microbial carbon determination. This comparison appears as a useful purpose to establish the contribution of the calorimetric method to estimate soil microbial activity. In present case, organic additives and a herbicide are employed in order to follow their influence on soil microbial activity, as occurred in some agricultural systems.

2. Experimental

2.1. Soils and soil sampling

Soil samples were taken from three different areas in the State of São Paulo, Brazil: a Rhodic eutrudox (R), a Typic eutrudox (V) and a Quartzipsamment (Q) [15]. The samples were collected at a depth of 5–10 cm, after removal of the litter on the soil surface [16]. The soil samples were amended with 25% of cattle manure (E), or municipal refuse compost (L) or earthworm casts (H) or with trifluralin (T) in a dose equivalent to 1.25 kg ha^{-1}. To all assayed samples 20% of water was added, while only water was added to the control.

The moisture content (%) was determined by drying the soil sample to a constant weight at 378 ± 2 K. The OM fraction of the soil sample was oxidized and determined by an oxy-reduction reaction. The pH was obtained in strong electrolyte, 1.0 mol dm^{-3} of calcium chloride solution, in a proportion of 1:2.5 for soil/solution. The total acidity (H + Al) was determined by percolating 5.0 g of dry fine soil in air with 0.10 dm^3 of 2.0 mol dm^{-3} calcium acetate at pH 7.0. The cation exchange capacity (CEC) and the total extractable bases (SB) were obtained by extracting the percolated fraction of 10.0 g of the soil with 0.10 dm^3 of 5.0×10^{-2} mol dm^{-3} nitric acid solution. Na^+ and K^+ were analyzed by flame photometry and determined through a previously obtained calibration curve. The cations Mg^{2+}, Ca^{2+} and Al^{3+} were determined through atomic absorption spectrometry [16].

The percentage of water (% WHC) in the soils was measured in tubes with known amount of soil and the volume of water relationship, to give a condition

of 60% of the maximum water holding capacity. All samples were maintained at 298 ± 2 K and the measurements were performed from the initial day considered as zero, followed by 7, 14, 21, 28, 42, 56, 70–91 days of incubation. All measurements were obtained in triplicate run [16].

2.2. Fumigation–extraction method

The microbial biomass C was determined by applying the proposed method [17]. The soil sample was maintained under refrigeration until the biomass was measured. The initial microbial biomass C was determined 24 h after sampling and at this moment received the amendments: E, L, H, or T. The control (A) was the soil sample containing water and the moisture content was determined for all these samples.

2.2.1. Carbon extraction

According to the established method [17], soil subsamples were fumigated with chloroform and incubated for 2 days at 303 ± 3 K.

The extraction of all the fumigated and non-fumigated samples was obtained with 0.50 mol dm^{-3} K_2SO_4 solutions in a proportion of 1:4 (soil-extractor) with stirring for 30 min, after which the samples were quantitatively filtered.

2.2.2. Determination of biomass carbon

The organic carbon oxidation method with a $Cr_2O_7^{2-}$ solution is represented by the reaction (1):

$$2Cr_2O_7^{2-} + 3C^0 + 16H^+ \rightleftharpoons 4Cr^{3+} + 3CO_2 + 8H_2O \quad (1)$$

For the sequential determinations, 4.0 cm^3 of soil extract were amended with 1.0 cm^3 of 0.066 mol dm^{-3} $K_2Cr_2O_7$ solution, 5.0 cm^3 of concentrated H_2SO_4 and 2.5 cm^3 of concentrated H_3PO_4. Simultaneously, a control with K_2SO_4 was also operated. The solutions were heated for 30 min and after cooling, the volume was completed to 25.0 cm^3 with distilled water. Titration was performed with diphenylamine and 0.033 mol dm^{-3} ammoniacal ferrous sulfate solution.

Microbial biomass C was determined by the Eq. (2):

$$B = \frac{C_F - C_{NF}}{K_{EC}} \quad (2)$$

C_F and C_{NF} are the amount of carbon extracted from fumigated and non-fumigated soil subsamples and K_{EC} is the constant for tropical soil and in the present case its value is 2.64.

2.3. Microcalorimetric method

The thermal effect on a microscale (mg or μg per sample) was measured in a model LKB 2277 isothermal calorimeter, to determine the variation of enthalpy of the system. Each thermal power value is determined and analyzed from the calorimetric curve. The calorimeter was calibrated by the release of electrical energy in a resistor of the instrument. The thermal effect of the sample ampoule was adjusted to the electrical calibration. The calorimetric has a precise control of the isothermal conditions in the thermostated bath and of the detection of the thermal events in the system [18,19].

The thermal effect was obtaining by using 5.0 cm^3 stainless steel ampoules, which were hermetically closed by Teflon sealing discs. This procedure was employed in order to control evaporation and transfer of oxygen and carbon dioxide [20]. The sequential determinations were carried out at 298.15 ± 0.02 K. All determinations were performed in ampoules containing 0.75 g of soil, 0.25 g of organic material and 0.25 cm^3 of aqueous solution, and 1.00 cm^3 distilled water was used in the reference ampoule. For T, 23 μg of this compound in aqueous solution were added to the sample [21]. After being thermostated, the thermal effect associated with microbial activity was recorded as function of time. The final value was calculated by comparing the integrated area of the power time curves, which correspond to the thermal effect of the experiment. All enthalpic values were obtained from triplicate run [19].

3. Results and discussion

3.1. Soils and organic material characteristics

The soil properties and those of the additives investigated are shown in Table 1. The lowest set properties for those values are found for natural Q soil. With exception of potential acidity all other properties increased with addition of organic compounds. Thus,

Table 1
Properties of soils: organic matter (OM), pH, phosphorus (P), exchangable cations (K, Ca, Mg), potential acidity (H + Al), extractable bases (SB) and cation exchange capacity (CEC) for Rhodic eutrudox (R), Typic eutrudox (V), Quartzipsamment (Q), cattle manure (E), municipal refuse compost (L) and earthworm casts (H)

	R	V	Q	E	L	H
OM ($g\,dm^{-3}$)	33 ± 2	23 ± 1	3.0 ± 0.2	114 ± 6	99 ± 5	122 ± 6
pH	5.0 ± 0.3	6.2 ± 0.3	4.7 ± 0.2	7.4 ± 0.4	7.5 ± 0.4	6.7 ± 0.3
P ($mg\,dm^{-3}$)	32 ± 2	73 ± 4	2.0 ± 0.1	643 ± 32	304 ± 2	712 ± 36
K ($mmol\,dm^{-3}$)	2.5 ± 0.1	1.1 ± 0.1	0.20 ± 0.01	76.8 ± 4	23.8 ± 1	19.3 ± 1
Ca ($mmol\,dm^{-3}$)	23 ± 1	46 ± 2	2.0 ± 0.1	91 ± 5	410 ± 21	113 ± 6
Mg ($mmol\,dm^{-3}$)	12 ± 1	26 ± 1	1.00 ± 0.05	155 ± 8	55 ± 23	62 ± 3
H + Al ($mmol\,dm^{-3}$)	38 ± 2	14 ± 1	11 ± 1	10 ± 1	8 ± 1	13 ± 1
SB (%)	51 ± 3	82 ± 4	23 ± 1	97 ± 5	98 ± 5	94 ± 5
CEC ($mmol\,dm^{-3}$)	76 ± 4	87 ± 4	15 ± 1	333 ± 17	497 ± 25	207 ± 10

the original acidic soil values change to neutral condition and as expected, a considerable increase in OM, P, K, Ca, Mg and CEC values were observed. Then, the organic additives masked the original chemical characteristic of these soils.

3.2. Evaluation of microbial biomass C by the fumigation–extraction method

Biomass values, expressed as the sum of C over the 0–91 days range of incubation time, are shown in

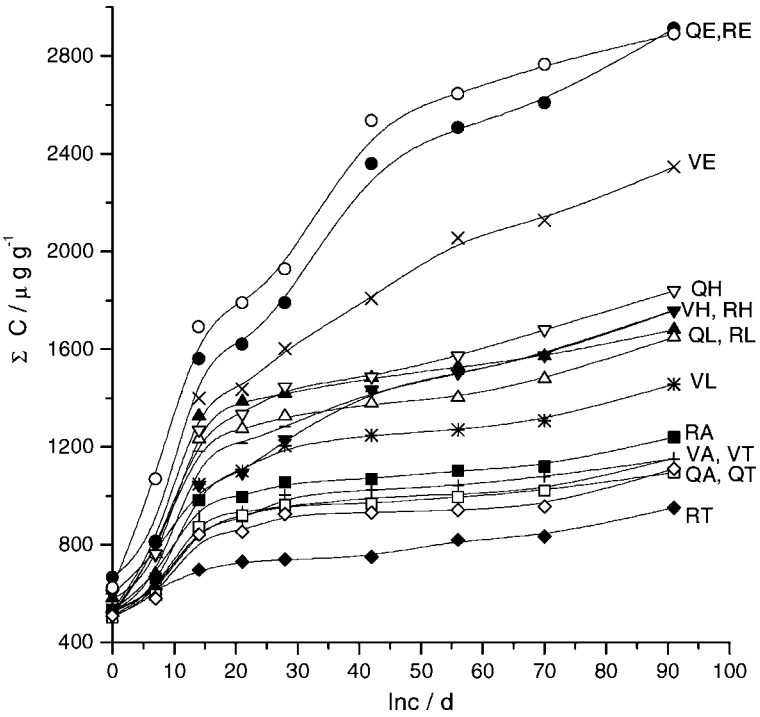

Fig. 1. Results of biomass carbon (C) as a function of incubation time (Inc, day) for Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) soils, for the control (A) and after amendment of 25% of cattle manure (E), or municipal refuse compost (L), or earthworm casts (H) or 23 μg of trifluralin (T), at 303 ± 3 K.

Fig. 1. The Tukey test for the results listed in Fig. 1 reveal no differences among the three soils and show that the chemical characteristics of the soils were not a decisive factor for microorganism development. On the other hand, the results imply also that the microbial biomass C method does not detect the differences in the characteristics of the soils studied.

Fig. 1 shows that E supports major microbial biomass C in all soils and a prominent effect in relation to L and H. The results of the sequential series of these materials are defined by C microbial biomass applied in these studies. The source of organic amendments was the major important factor to affect the microbial biomass C. Cattle manure provided labile compounds for different microbial populations in the soil. The sequential E, H and L results listed in Table 2 imply better conditions for the development of the largest population. Different organic additives imply differences in microorganism growth and consequent differences in measurements of microbial biomass. It is probable that the substrate amended into the soil for microbial response is not available to the majority of soil microorganisms. It is also evident that the microbial biomass C technique did not detect any difference between the control and when T is presented.

The results shown in Fig. 1 for the first sampling, in the control treatment, the R and V soils had the highest value for biomass C and Q soil had the lowest one. At the same sampling the relative positions of the treatments varied with the soil. In the sequential series shown in Fig. 1 it is possible to observe variations in all samplings. These differences can be attributed not only too chemical characteristics of each soil but also to the different microbial communities, which result from the chemical characteristics of each soil. For instance, in the treatments with E, the soils had the highest values of microbial biomass C and these results increased significantly in relation to the other amendments, after a period of 10 days of incubation.

The results in Fig. 1 show higher values for Q soil for all amendments, even though R and V soils have higher OM and nutrients quantities, as listed in Table 1. It is possible that the R and V soils can retain the organic carbon for utilization as reserves for future activities. This result can be related with the health of the soil and the storage of organic material, as previously observed [22]. The quality of soil is an important characteristic to sustain the biosphere and agriculture. This definition has been presented in different ways as bioproductibility, sustentability, and environment protection for human and animal health [23].

3.2.1. Microbial biomass C per day of incubation time

The illustration of the results of microbial biomass C ($\mu g\, g^{-1}$ per day) as a function of incubation time (day) plot is shown in Fig. 2. The profiles of curves for three soils are similar: the highest values appeared in the initial part of curve and a decrease is observed in the period of 91 day of incubation. This fact can be associated with the organic material introduced, which enhances the microbial activity. The microbial communities had the ability to degrade the different organic compounds amended to the soils, with different carbon sources. The results of these processes are observed through the highest respiration effects [24] and by increasing the microbial biomass C. At the end of the period of incubation a plateau is reached for all curves. The values had a slow decrease and tend to a similar stage, indicating that the microbial processes are stabilized. This fact is related with the decrease in the microbial degradation of organic material and a consequent decrease in growth. It is expected also that in the incubation period a modification occurs in the conditions of development of the microbial communities that were active. Thus, other populations were stimulated to use the by-compounds degraded by the initial populations and to provide material for the biological carbon cycle.

3.3. Calorimetric results and comparison with the microbial biomass C method

The calorimetric curves obtained for all systems investigated are shown in Fig. 3. Thus, the sum of the calorimetric values in the period of the incubation time of 103 days is illustrated. The thermal effect resulted from the area of the curve ΔP versus the respective time. These results showed the decreasing sequence: E, H, L and T. The thermal effects in these systems reflect the degradation of the organic materials amended by community, present in each soil, as observed before [20]. These results can be associated with the amount of active organic compounds present in the materials, where the E that was enriched with large amounts of labile organic material, caused the

Table 2
The average *M*(row, Mc; column, M_L; all experiments, M_T) microbial biomass C ($\mu g\,g^{-1}$) values for Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) soils with 20% of water (A) and 25% of cattle manure (E), municipal refuse compost (L), earthworm casts (H) and trifluralin (T) at time (t) at 303 ± 3 K

t per day	Soil	A	E	L	H	T	M_L
0	R	552.80^{Abc}	1329.25^{Aa}	574.90^{Abc}	592.65^{Ab}	519.78^{Ac}	713.88^{A}
	V	521.07^{ABc}	1064.20^{Ca}	506.06^{Bc}	623.61^{Ab}	520.13^{Ac}	647.01^{B}
	Q	484.28^{Bb}	1242.21^{Ba}	515.61^{Bb}	502.91^{Bb}	533.08^{Ab}	655.62^{B}
Mc		519.38^{c}	1211.88^{a}	532.19^{c}	573.06^{b}	524.33^{c}	672.17
7	R	130.11^{Abc}	315.15^{Ca}	112.77^{Bbc}	160.54^{Bb}	92.64^{Ac}	162.24^{B}
	V	75.42^{Ab}	497.71^{Ba}	112.33^{Bb}	123.93^{Bb}	76.27^{Ab}	177.13^{B}
	Q	91.58^{Ac}	894.45^{Aa}	239.57^{Ab}	254.88^{Ab}	73.70^{Ac}	310.83^{A}
Mc		99.04^{c}	569.10^{a}	154.89^{b}	179.78^{b}	80.87^{c}	216.74
14	R	320.76^{Ab}	698.15^{Aa}	644.48^{Aa}	283.99^{Bb}	81.14^{Bc}	405.70^{B}
	V	287.38^{Ad}	623.77^{Ba}	446.70^{Bc}	549.39^{Ab}	313.57^{Ad}	444.16^{A}
	Q	277.65^{Abc}	116.22^{Cd}	338.93^{Cb}	505.86^{Aa}	267.51^{Ac}	301.23^{C}
Mc		295.26^{b}	479.38^{a}	476.70^{a}	446.41^{a}	220.74^{c}	383.70
21	R	10.28^{Aa}	59.44^{Ba}	58.06^{Aa}	49.17^{Aa}	32.95^{Aa}	41.98^{AB}
	V	20.09^{Aa}	38.12^{Ba}	56.76^{Aa}	33.34^{Aa}	16.94^{Aa}	33.05^{B}
	Q	48.50^{Ab}	128.68^{Aa}	42.13^{Ab}	64.49^{Aab}	11.68^{Ab}	59.10^{A}
Mc		26.29^{b}	75.42^{a}	52.32^{ab}	49.0^{ab}	20.53^{b}	44.71
28	R	59.18^{Bb}	169.75^{Aa}	35.60^{Bb}	137.19^{Aa}	6.93^{Bb}	81.73^{A}
	V	116.56^{Aab}	165.03^{Aa}	102.92^{Aab}	68.24^{Bbc}	27.79^{ABc}	96.11^{A}
	Q	41.81^{Bc}	156.76^{Aa}	53.10^{ABbc}	113.42^{ABab}	72.82^{Abc}	87.58^{A}
Mc		72.52^{bc}	163.85^{a}	63.88^{c}	106.28^{b}	35.85^{c}	88.47
42	R	12.31^{Ac}	570.04^{Aa}	76.49^{Ac}	215.12^{Ab}	58.70^{Ac}	186.53^{A}
	V	19.61^{Ab}	207.58^{Ba}	42.86^{Ab}	144.59^{Ba}	36.60^{Ab}	90.25^{C}
	Q	19.33^{Ab}	607.58^{Aa}	50.88^{Ab}	42.83^{Cb}	7.10^{Ab}	145.54^{B}
Mc		17.08^{d}	461.74^{a}	56.74^{c}	134.18^{b}	34.13^{cd}	140.77
56	R	35.19^{Ab}	148.58^{Ba}	43.22^{Ab}	71.75^{Ab}	68.94^{Ab}	73.54^{A}
	V	27.30^{Abc}	247.22^{Aa}	24.08^{Abc}	82.11^{Ab}	16.39^{ABc}	79.42^{A}
	Q	38.69^{Abc}	109.89^{Ba}	33.61^{Abc}	84.17^{Aab}	10.29^{Bc}	55.33^{A}
Mc		33.72^{c}	168.56^{a}	33.64^{c}	79.34^{b}	31.87^{c}	69.43
70	R	15.42^{Ac}	99.96^{ABab}	42.81^{Bbc}	71.16^{Abc}	146.70^{Aa}	75.29^{A}
	V	34.60^{Aa}	77.56^{Ba}	37.52^{Ba}	65.21^{Aa}	17.18^{Ba}	46.41^{B}
	Q	26.50^{Ac}	139.98^{Aa}	105.03^{Aab}	106.46^{Aab}	62.17^{Bbc}	88.03^{A}
Mc		25.51^{d}	105.83^{a}	61.79^{c}	80.94^{b}	75.35^{bc}	69.91
91	R	120.40^{Abc}	303.57^{Aa}	110.93^{Bc}	184.16^{Ab}	118.54^{Ac}	167.52^{A}
	V	71.73^{Ac}	219.87^{Ba}	148.70^{ABb}	178.38^{Aab}	133.31^{Abc}	150.40^{AB}
	Q	73.60^{Ab}	124.88^{Cab}	171.83^{Aa}	160.10^{Aa}	154.37^{Aa}	136.96^{B}
Mc		88.58^{e}	216.11^{a}	143.82^{d}	174.21^{b}	135.41^{d}	151.62^{c}
M_T	R	139.59^{Ac}	410.43^{Aa}	188.81^{Ab}	196.21^{Ab}	125.15^{Ac}	212.04^{A}
	V	130.42^{Ad}	349.01^{Ca}	164.21^{Bc}	207.64^{Ab}	128.69^{Ad}	195.99^{B}
	Q	122.44^{Ad}	391.18^{Ba}	172.30^{ABc}	203.90^{Ab}	132.52^{Ad}	204.47^{A}

The same small letter on the numbers in a row and the columns indicate no statistical differences (Tukey < 0.05).

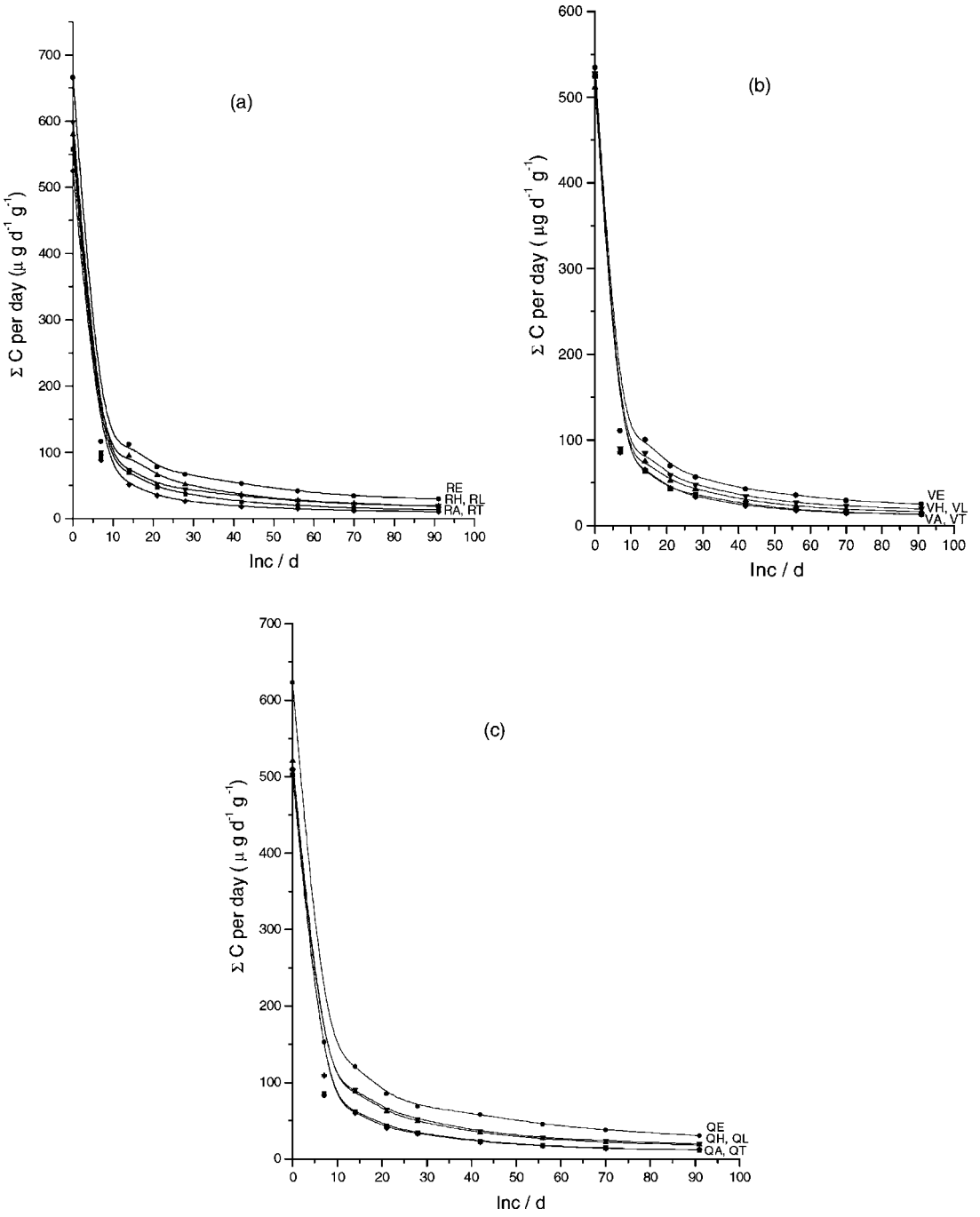

Fig. 2. Sum of biomass carbon (C) per day of incubation (Inc) for Rhodic eutrudox (a), Typic eutrudox (b) and Quartzipsamment (c) soils for the control (A) and after amendment of 25% of cattle manure (E), or municipal refuse compost (L), or earthworm casts (H) or 23 μg of trifluralin (T), at 303 ± 3 K.

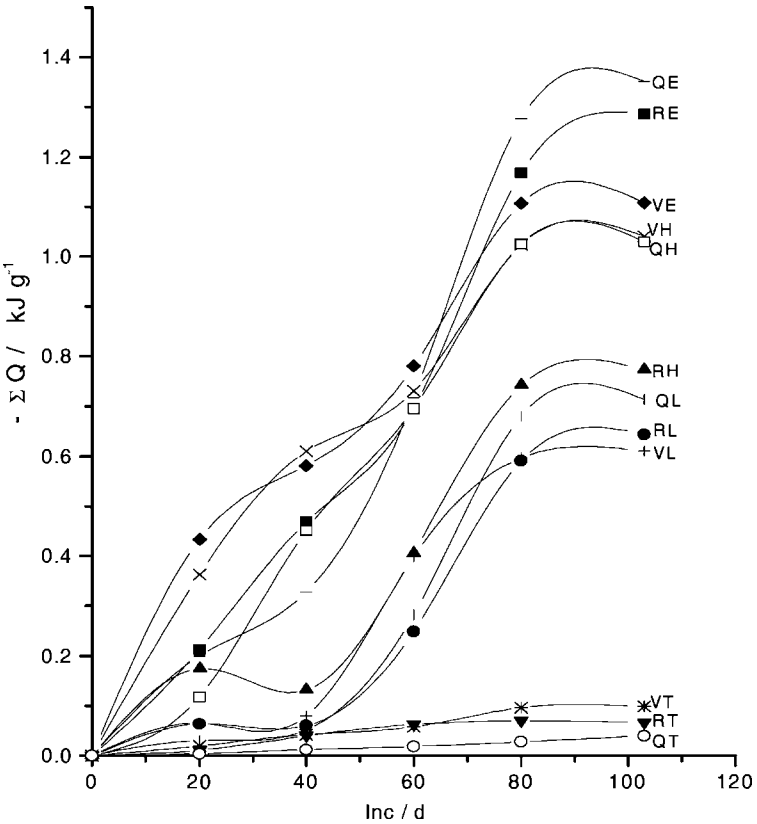

Fig. 3. Calorimetric curves over 103 days of incubation time for all soil samples studied at 298.15 ± 0.02 K.

highest thermal effect. The curves at the end of 103 days reach a slight plateau. This can imply in a stationary state with minor microbial activity.

The correlation between microbial biomass C and the thermal effect obtained by the calorimetric method is shown in Fig. 4. The correlation coefficient, $r = 0.7443$, and the statistic probability of occurrence of the event $P < 0.0001$, between the thermal effect and C microbial biomass, indicated a good correlation between both methods, because P values have 99.99% of confidence in the occurrence of the event. On the other hand, the results of microbial biomass C do not show the contribution of the total interactive effect of active microbiota in soil. Biomass C denotes only the total mount of carbon in the soil and does not necessarily reflect activities. The biomass C measurement [25] does not require soil fractionation to obtain the soil biota as is recommended for several indirect techniques, and in the end, all of them give close values [26]. Thus, the microbial biomass C does not reflect the competition of microbial populations and the inhibitory effects of metabolites. However, the thermal effect also reflected the interactive effect in the soil microenvironment and other characteristics of the process, like enzymatic reactions and thermal degradation of compounds available for growth.

In the course of this field of investigation a better correlation between respiration and thermal effect [24] was obtained. This behavior strongly suggests that a considerable fraction of amended organic material was available for microbial attack, which is lost as carbon dioxide and the combustion process can liberate higher quantities of thermal effect in the microbial growth. On the other hand, the correlation between microbial biomass C and thermal effect expresses the percentage of carbon retained in soil as microbial biomass.

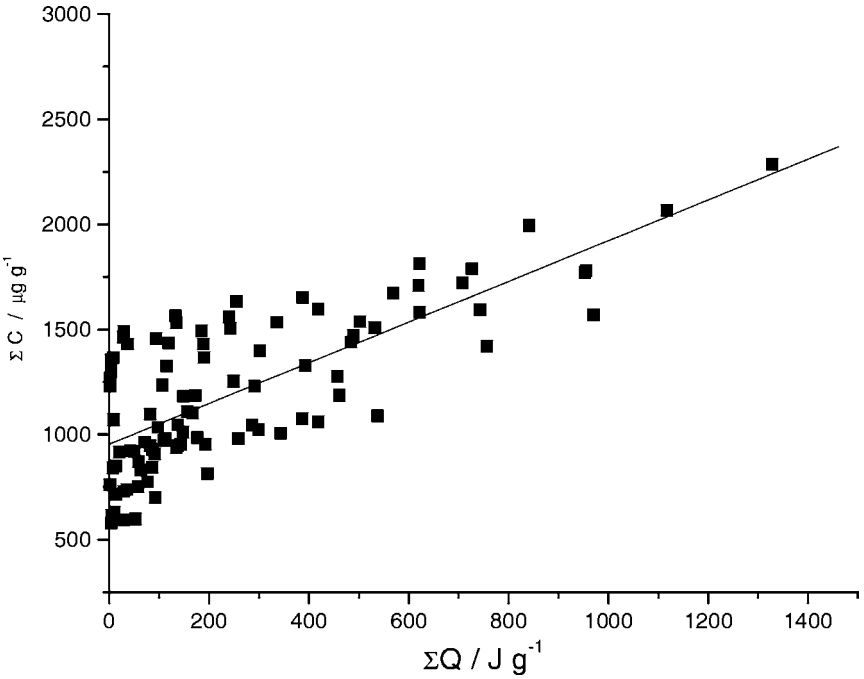

Fig. 4. Correlation between microbial biomass C and thermal effect for all tropical soils samples studied over 91 days.

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