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Comparison between microorganism counting and a calorimetric method applied to tropical soils

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Abstract

Bacteria and fungi, the major sources of microorganisms found in soil, play an essential role in nutrient degradations. Both kinds of microorganisms were quantitatively evaluated to provide a correlation between thermal effects and the respective number of microorganisms in the system. The microorganisms were quantified in agar plate counts and the thermal effect was calorimetrically determined in soil samples. The experiments were performed on tropical soils: a Rhodic eutrudox (R), a Typic eutrudox (V) and a Quartzipsamments (Q) from Brazil. The soils were amended with a range of organic materials: 25% of cattle manure, or municipal refuse compost, or earthworm casts, or 23 μg of an agrochemical, trifluralin (doses of 1.25 kg ha^{-1}) during incubations of 85 days. The results of simultaneous application of the two methods to measure microbial activity showed the correlation with $r = 0.8181$ and $P = 0.0131$ for bacteria and $r = 0.8134$ and $P = 0.014$ for fungi, over the period studied. The microbial activity decreased in the order: cattle manure, earthworm casts, municipal refuse compost and trifluralin.

Keywords: Tropical soils; Microorganism; Bacteria; Fungi; Microcalorimetry; Microbial activity

1. Introduction

The soil environment contains a large variety of microbes, which reflect the habitat, and the relative ability of the individual microorganisms to compete for the available nutrients. The majority of soil microbes require a supply of organic material, which may be obtained from a variety of carbon sources. However, a defined system with soil enriched with organic materials permits the development of a large number of distinct organisms. The bacteria and fungi populations represent a part of microbial community with high growth at large substrate concentrations. The counting of microorganisms can indicate their contribution to the natural environment, since they have an important role in fundamental chemical and biological processes in soil fertility and plant growth [1]. Thus, microbial growth evaluation is a reasonable model for microbial activity investigations in a given system.

The microbial growth involves an increase in the number of cells and the microorganisms are able to influence the environment through the products of their metabolism. In this context, the existence of microorganisms can be influenced by enzyme activities. These enzymes can be liberated from the membranes of microorganism cells to attack the organic molecules of soil, in order to break them down into a simpler product [2]. The activity of the soil microbiota has often been linked with the number of microorganisms,

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enzyme activities and microbial biomass. The evaluation and contribution of all these parameters is an important feature related to the metabolisms that have been well-investigated [3].

Knowledge of the dynamics of soil microorganisms presents many problems due to the complex and dynamic nature of soil. The vast quantities and varieties of organic material which exist in soil, the large surface for colonization and the continuous variation in environment conditions, result in the development of many distinct ecological niches. Also, the study of a particular technique widely used for enumerating colonies by microbiologists, the conventional plate count method, where the microorganisms are grown on culture media, and the resulting visible colonies counted, has some limitations. So, its comparison with the calorimetric method, that measures the contribution of total microbial activities, can offer advantages in order to understand a particular situation of microbial growth in the soil. Undoubtedly, the comparison of two techniques can indicate different aspects of microbial activity. But it is expected that the organisms, which are able to degrade the organic material, grown in large number are responsible for the majority of the thermal effect. On the other hand, the importance of such data is focused on the elucidation and interpretation of calorimetric results, although the growth reflects the dominant communities of microorganisms in the soil. The microbial activity can be evaluated by the thermal effect, which results from all living cell metabolisms [3].

The heterogeneity of the microorganisms themselves has a magnitude greater than the heterogeneity of perspectives from which they are contemplated by human observers. Even closely related species may exhibit marked differences in biochemistry and behavior [4]. The heterogeneity of microbial communities in the natural environment depends on possible features related to environmental variations, such as temperature, moisture content, salinity, pH, and dissolved oxygen and carbon concentrations. These environmental fluctuations obviously have a strong selective effect and may play a vital role in controlling the microbial activity [1].

Estimation of the total microorganism number in an aerobic sample depends on the handling of the sample and the analytical method employed [5]. Thus, by using methods of microscopy, like microspectrophotometry and cytometry, developed and refined since the 1960s, it is now possible to characterize the physiology and pharmacology of individual microorganisms. In many cases, this has enabled organism isolation with selected characteristics for culture and/or further analysis. Fluorescence measurements are particularly important in single-cell nucleic analysis, then, the data allow demonstration and quantification of cell nucleic acid contents and also the sequence, the presence of specific antigens, and physiological characteristics such as enzyme activity and membrane potential [4].

There is a widespread need for commercial instrumentation for quick and inexpensive detection of microbial contamination of food, industrial wastewater and clinical samples. Using optical, electrochemical, biochemical and physical properties of the microorganisms, a large number of detection methods have been developed. The need for a device which can produce a rapid, accurate, sensitive, real-time analysis for clinical, industrial and environmental applications has led to considerable progress being achieved in recent years in the development of biosensors for microbial detection [6].

Voltammetric measurements of microbial populations are possible with a modified glassy carbon electrode and the construction of the biosensor and an electrochemical cell with an elastic base. The limit of determination of the microbial populations was an average (2–5) $\times$ 10^4 cells with cyclic voltammetry and a measuring period of 30 min for a sample. The measurements showed that the method performed in a rapid and practical way for the determination of microbial populations in food and environmental sample [7].

The aim of this study is to investigate the correlation between the calorimetric data and the number of bacteria and fungi as a quantification of the microbial activity counted during 85 days. Simultaneously, the thermal effect on amended soil samples was measured, with some organic materials used in agriculture: cattle manure, municipal refuse compost or earthworm casts. The effects of trifluralin were also investigated due to its significant application in weed control in Brazilian agriculture. The thermal effect and the number of microorganisms are now reported in order to establish a comparison of these important data.

2. Materials and methods

2.1. Soils and sampling

Soil samples were taken from three different areas in the state of São Paulo, Brazil, which are a Rhodic eutrudox (R), a Typic eutrudox (V) and a Quartzipsamment (Q) [8]. Samples of 100.0 g were amended with 25% of additives: cattle manure (E), or municipal refuse compost (L), or earthworm casts (H), or trifluralin (T) and then incubated. An identical sample of 100.0 g was used as control, containing only 20% water (A). All operations were done at 298 ± 3 K. The samples were collected at a depth of 5–10 cm, after removal of litter on the soil surface, homogenized by sieving to less than 2 mm, to separate roots and large particles, and stored in polyethylene bags at 298 ± 3 K [9,10].

The percentage of moisture was determined by drying the sample to a constant mass at 383 ± 3 K. The organic matter (OM) was obtained by titrating the samples in an acidic medium, with the end point being followed by a redox reaction. The pH was measured in a strong electrolyte, 1.0 mol dm^{-3} calcium chloride solution, in a proportion of 1:2.5 for soil/solution. The total acidity ($H^+ + Al^{3+}$) 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 sum of extractable bases (SB) were obtained by extracting a 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, using a previously obtained calibration curve. Mg^{2+}, Ca^{2+} and Al^{3+} were determined through atomic absorption spectrometry [9,11].

The percentage of water (WHC, %) in the soils was measured in tubes with a known relationship between the amount of soil and the volume of water, for reaching 60% of the maximum water holding capacity [9].

2.2. Analysis of microbial population

The numbers of microorganisms in the soil samples were analyzed immediately after collection by the agar dilution method. A sample of 10.0 g of soil and 90.0 cm^3 of sterilized water were stirred for 30 min. The resulting soil suspensions were diluted in factors of 10 and aliquots were spread in appropriate culture media. The soil extract medium was used to grow bacteria and Martin medium for fungi [12]. They were incubated at 303 ± 2 K and after 2 days the colony forming units (cfu) were counted in the plates, varying from 30 to 300 [13]. All counting was carried out on five replicates.

2.3. Microcalorimetry

The thermal effect on a microscale (mg or μg per sample) was measured in a model LKB 2277 isothermal calorimeter to determine the variation enthalpy of the system. Each thermal power value was determined and analyzed from the calorimetric curve. The calorimeter was calibrated by the release of electrical energy in a resistor of the instrument and the thermal effect of the sample ampoule was adjusted to the electrical calibration. This calorimeter has a precise control of the isothermal conditions in the thermostated bath and the detection of the thermal events in the system [14,15].

The thermal effect was obtaining by using 5.0 cm^3 stainless steel ampoules, which were hermetically closed by Teflon sealing discs. This adopted procedure was established in order to control evaporation and transfer of oxygen and carbon dioxide [16]. In all determinations the ampoules contained 0.75 g of soil, 0.25 g of organic material: cattle manure (E), or municipal refuse compost (L), or earthworm casts (H), or trifluralin (T), plus 0.25 cm^3 of distilled water, and only water was used for the reference ampoule at 298.15 ± 0.02 K. After thermostating, the thermal effect associated with degradation was recorded as a function of time. The final value was calculated by comparing the integrated area of the power time curves, which corresponds to the thermal effect of the experiment [17]. All enthalpic values were obtained from triplicate runs.

3. Results and discussion

3.1. Soils and organic compound characteristics

Table 1 summarizes the results obtained for chemical analysis for soils and organic amendments, which showed easily distinguishable values for crude soils

Table 1
Characteristics of soils amended with organic matter (OM), pH, phosphorus (P), exchange cations (K, Ca, Mg), potential acidity (H + Al), sum of bases (SB) and cation exchange capacity (CEC) for the 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

and those which were amended. An inspection of these data shows that Q soil had the lowest values for all listed. These three original soils, R, V and Q, are acidic in nature and the SB values are very low for Q, medium for R and high for V soils. However, when the organic compounds were added higher values for OM, P and CEC values, with neutral pH and much higher SB values were detected [18].

3.2. Number of microorganisms

The results of fungi and bacteria counting are shown in Tables 2 and 3, respectively.

The stimulation of fungi populations was less pronounced than that of bacteria. The Rhodic eutrudox (R) have more microorganisms in relation to the Typic eutrudox (V) and Quartzipsamment (Q). Original Q soil presented smaller microorganisms communities and the lowest properties in comparison with the other two soils, as listed in Table 1. This set of results indicated that Q soil is associated with some factor of stress. The application of organic material in general significantly increases the population of bacteria and fungi in all soils [19]. The amounts added are three times higher than agricultural doses, which are applied to soil without amendments. This dose could mask the chemical properties of the soils, but in lesser intensity than their physical properties. Then, physical factors can favor in larger intensity the development of microbial communities in the R soil, when compared to V and Q soils [20].

The cattle manure significantly increases the number of fungi cfu in the soil, but the other amendments did not differ from the control, as shown in Table 2. For the bacteria, the cattle manure showed the highest values, but the municipal refuse compost and earthworm casts also differed from the control and that of trifluralin treatments.

The capacity of cattle manure in supporting microbiota, in this case, the bacteria and fungi, overcomes the capacity of other organic materials. This fact indicates greater utilization of compounds present in cattle manure as well as their degraded products by the soil microorganisms, to derive energy and nutrients for their cellular metabolism over a period of 3 months. The cattle manure showed an effective degradation by microorganisms. These soils containing organic materials named E, L and H have distinct compositions, which influence the decomposition processes under the same environmental conditions. Then, this effect of the cattle manure E indicated that a great fraction of this material is composed of materials easily degradable by soil microbiota, as shown by the media values found in rows and columns for all incubation times in Tables 2 and 3. This behavior also indicates a significant amount of labile organic matter in cattle manure, in relation to the other organic materials investigated. It is possible that the municipal refuse compost and earthworm casts have a greater portion of resistant organic matter, not to be attacked by microorganisms, during the time of the experiment [20].

The number of fungi in Table 2 was shown to be insensitive to trifluralin. This fact indicated that the trifluralin tested at the recommended field dose had no deleterious effect on the growth and activities of the counted fungi in soil. The number of bacteria in

Table 2
Fungi number ($\times 10^3\ g^{-1}$) and average M values (row M_L, column M_C and M_T for all experiments) in dry soil samples of Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) with control (A), cattle manure (E), municipal refuse compost (L), earthworm casts (H), and trifluralin (T) at different incubation times (Inc), at 301 ± 3 K[a]

Inc (days)	Soil	Amendments					M_C
		A	E	L	H	T	
1	R	5.64 Ab	13.40 Ba	8.13 Ab	5.26 Ab	6.73 Ab	7.80 A
	V	1.63 Bb	7.37 Ca	0.46 Bb	0.56 Bb	0.56 Bb	2.12 C
	Q	0.20 Bb	16.60 Aa	0.30 Bb	0.99 Bb	0.26 Bb	3.67 B
	M_L	2.49 b	12.46 a	2.96 b	2.27 b	2.52 b	
15	R	2.91 Ab	73.40 Aa	6.94 Ab	3.34 Ab	2.15 Ab	17.75 A
	V	1.43 Ab	19.43 Ba	5.63 Ab	5.99 Ab	1.48 Ab	6.79 B
	Q	0.39 Ab	17.52 Ba	0.94 Bb	0.75 Bb	0.12 Ab	3.94 B
	M_L	1.58 b	36.78 a	4.50 b	3.36 b	1.25 b	
29	R	2.32 Ab	71.13 Aa	8.60 Ab	4.49 Ab	5.08 Ab	18.32 A
	V	1.76 Ab	28.34 Ba	4.46 Abb	2.08 Ab	2.00 Abb	7.73 B
	Q	0.13 Ab	23.01 Ca	0.15 Bb	1.18 Ab	0.12 Bb	4.92 C
	M_L	1.40 b	40.83 a	4.40 b	2.58 b	2.40 b	
43	R	1.68 Ab	91.40 Aa	0.92 Ab	3.89 Ab	4.56 Ab	20.49 A
	V	1.83 AB	42.50 Ba	1.29 Ab	0.80 Bb	1.33 Abb	9.55 B
	Q	0.11 Ab	34.20 Ba	0.24 Ab	0.45 Bb	0.89 Bb	7.18 B
	M_L	1.21 b	56.03 a	0.82 b	1.71 b	2.26 b	
57	R	2.24 Ab	88.00 Aa	1.96 Ab	2.08 Ab	1.86 Ab	19.23 A
	V	1.54 Ab	46.00 Ba	1.14 Ab	1.22 Ab	1.20 Ab	10.22 B
	Q	0.13 Ab	23.86 Ca	0.19 Ab	0.55 Ab	0.10 Ab	4.97 C
	M_L	1.30 b	52.62 a	1.10 b	1.28 b	1.05 b	
71	R	1.88 Ab	79.99 Aa	6.43 Ab	1.59 Ab	1.53 Ab	18.28 A
	V	1.43 Ab	23.83 Ba	1.20 Ab	0.83 Ab	1.72 Ab	5.80 B
	Q	0.10 Ab	27.81 Ba	0.19 Ab	0.55 Ab	0.06 Ab	5.72 B
	M_L	1.14 b	43.88 a	2.61 b	0.99 b	1.10 b	
85	R	5.08 Ab	63.10 Aa	5.60 Ab	1.76 Ab	1.94 Ab	15.50 A
	V	1.01 Ab	29.08 Ba	1.30 Ab	1.19 Ab	0.56 Ab	6.76 B
	Q	0.16 Ab	10.95 Ca	0.07 Ab	0.21 Ab	0.26 Ab	2.2 C
	M_L	2.08 b	34.38 a	2.32 b	1.05 b	0.92 b	
	M_T	1.60 b	39.57 a	2.67 b	1.89 b	1.65 b	
	R	16.77 A					
	V	6.99 B					
	Q	4.66 C					

[a] The number associated with the same small letter in the columns and the same capital letter in the rows shows no statistical differences (Tukey < 0.05).

the trifluralin counting sample from Table 3 showed a lesser value than the control. These results indicate that the communities cannot use the great fraction of nutrients in the soil, for supporting their growth in the presence of this pesticide dose.

The municipal refuse compost and earthworm casts had similar results, which reflect the characteristics of these organic materials. It is possible that the compounds present in earthworm casts may already have suffered a microbial decomposition, resulting in few decomposable compounds, with slow degradation, which require a special microbiota, with only a few individual species present in the soil. On the other hand, the municipal refuse compost has large quantities of nutrients and also OM as shown in Table 1. However, this composition has higher quantities of

Table 3
Bacteria number ($\times 10^3\,g^{-1}$) and average M values (row M_L, column M_C and M_T for all experiments) in dry soil samples of Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) with control (A), cattle manure (E), municipal refuse compost (L), earthworm casts (H), and trifluralin (T) at different incubation times (Inc), at $301 \pm 3\,K$[a]

Inc (days)	Soil	Amendments					M_C
		A	E	L	H	T	
1	R	0.07 Ac	1.40 Bb	0.42 Ac	5.28 Aa	0.62 Ac	1.56 A
	V	0.45 Aa	0.72 Ba	0.46 Aa	0.80 Ba	0.13 Aa	0.51 B
	Q	0.07 Ab	3.6 Aa	0.001 Bb	0.71 Bb	0.01 Ab	0.91 AB
	M_L	0.20 b	1.93 a	0.33 b	2.26 a	0.25 b	
15	R	0.09 Ac	4.76 Aa	1.07 Abc	1.17 Aab	0.21 Abc	1.46 A
	V	0.76 Ab	2.85 ABa	1.23 Aab	1.30 Aab	0.17 Ab	1.26 A
	Q	0.01 Aa	1.54 Ba	0.14 Aa	0.67 Aa	0.01 Aa	0.47 B
	M_L	0.29 bc	3.05 a	0.81 bc	1.05 b	0.13 c	
29	R	1.55 Ab	1.65 Bb	5.26 Aa	1.08 Bb	1.67 Ab	2.24 A
	V	1.29 Ab	8.20 Aa	1.76 Abb	2.17 Ab	1.44 Ab	2.97 A
	Q	0.02 Aa	0.99 ba	0.85 Ba	1.48 Ba	0.03 Aa	0.67 B
	M_L	0.95 b	3.61 a	2.62 a	1.58 ab	1.05 b	
43	R	0.74 Ab	1.15 ABb	0.58 Ab	2.25 Aa	0.77 Ab	2.49 A
	V	0.61 Aa	1.34 Aa	0.58 Aa	0.97 Ba	0.51 Aa	0.52 B
	Q	0.004 Ab	0.23 Bb	0.02 Ab	2.33 Aa	0.01 Ab	0.52 B
	M_L	0.45 b	0.91 ab	0.39 b	1.85 a	0.43 b	
57	R	1.51 Ab	9.46 Aa	2.22 Ab	3.33 Ab	1.16 Ab	3.54 A
	V	0.64 Ab	5.13 Ba	1.35 Aab	2.69 Aab	0.89 Ab	2.14 B
	Q	0.04 Ab	6.65 ABa	167 Ab	1.87 Ab	0.02 Ab	2.05 B
	M_L	0.73 bc	7.08 a	1.75 bc	2.63 b	0.69 c	
71	R	1.62 Ab	21.57 Aa	3.29 Ab	2.42 Ab	1.50 Ab	6.08 A
	V	0.99 Aa	2.45 Ca	1.78 Aa	1.85 Aa	0.64 Aa	1.54 B
	Q	0.01 Ab	7.41 Ba	1.07 Ab	1.84 Ab	0.01 Ab	2.07 B
	M_L	0.87 b	10.48 a	2.05 b	2.04 b	0.72 b	
85	R	1.06 Aa	2.77 ABa	1.38 Aa	1.45 Aa	0.95 Aa	1.52 A
	V	1.11 ABa	1.57 Ba	0.64 Aa	0.59 Aa	0.59 Aa	0.90 A
	Q	0.01 Bb	4.48 Aa	0.77 Ab	1.01 Ab	0.004 Ab	1.25 A
	M_L	0.73 ab	2.94 a	0.93 ab	1.02 ab	0.51 b	
	M_T	0.60 c	4.29 a	1.27 b	1.78 b	0.54 d	
	R	2.70 A					
	V	1.44 B					
	Q	0.37 C					

[a] The number associated with the same small letter in the columns and the same capital letter in the rows show no statistical differences (Tukey < 0.05).

synthetic plastic material, glass and other materials, which are unexpected to be decomposed in short time through microbial activity [20].

In general, the number of microorganisms in soils treated with different organic materials was higher when compared with that of the control, with the exception of the trifluralin compound. This fact indicated that organic materials stimulated the growth of microorganisms that degraded these compounds to form cellular carbon. During the incubation time of 3 months, it is possible that the occurrence of successive communities were benefited by the microbial activity that acts in the initial phase of organic materials degradation. The breakdown of any substrate reaching the soil may be regarded as a two-stage process. In the first one, the more readily assimilated substances

are quickly used up and the microbes return to their original state of low activity. In the second stage, the remaining materials, consisting of both added substrates and the products of the recent communities, are more resistant to decomposition and, consequently, a less intense microbial activity is observed [20].

A linear correlation between these data, by plotting the logarithm of microorganisms number (N) versus the incubation time (t), was obtained, as illustrated in Figs. 1 and 2. Then, it is assumed that the change in $\ln N$ as a function of time expresses the microbial growth in the soil and is also proportional to the change in incubation time, as represented by the following equation:

$$\ln \sum N(t) = \alpha t + \ln N(0)$$

This model was conveniently adjusted to the number of organisms for an incubation time (days), applied to the sum of microorganisms in a given time $\ln \sum N(t)$, $\ln N(0)$ being the number of them at the initial time of experiments, when the soil sample was amended, and α is the angular coefficient (g^{-1} per day), whose value can be determined by applying a linearization of this equation. Thus, such results, derived from this equation are shown in Table 4.

From the data listed in Table 4 it is clear that α values are directly dependent on fungi and bacteria growth in cattle manure. The rate of growth for fungi in municipal refuse compost was higher than in earthworm casts for RL, VL and in RL and QL for bacteria. The values in Table 4 for VA fungi and RA bacteria were higher and correspond to greater microbial activity. The inhibitor effect for growth rate in trifluralin/soil was present only for Quartzipsamment soil. The change in the microbial activity with incubation time was previously observed, when copper sulfate was applied to soil and followed through microcalorimetric method [21].

Table 4
The parameters ln(0) and α of linear regression for $\ln \sum N(t) = \alpha t + \ln N(0)$, for bacteria and fungi isolated from soil samples of Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) with control (A), cattle manure (E), municipal refuse compost (L), earthworm casts (H) and trifluralin (T) for 85 days of incubation time and the respective coefficient of correlation (r) and statistics P, at 301 ± 3 K

Soil	ln(0)	α	r	$P \times 10^{-3}$
Bacteria number				
RA	14.31	0.42	0.98	<0.10
RE	16.88	0.61	0.98	<0.10
RL	15.06	1.07	0.82	2.51
RH	16.50	1.01	0.98	<0.10
RT	14.01	0.42	0.98	<0.10
VA	14.26	0.89	0.99	<0.10
VE	15.94	1.27	0.99	<0.10
VL	14.52	0.96	0.99	<0.10
VH	14.87	0.96	0.98	<0.10
VT	14.02	0.88	0.99	<0.10
QA	11.14	0.62	0.98	<0.10
QE	15.06	1.47	0.99	<0.10
QL	13.16	1.48	0.96	0.48
QH	13.94	1.42	0.99	<0.10
QT	11.66	0.44	0.97	0.42
Fungi number				
RA	8.77	0.83	0.96	<0.10
RE	11.11	1.48	0.98	0.12
RL	9.29	1.11	0.94	1.41
RH	8.95	0.88	0.95	1.09
RT	8.81	0.95	0.94	1.76
VA	8.31	0.66	0.99	<0.10
VE	9.69	2.10	0.99	<0.10
VL	9.04	0.42	0.96	0.71
VH	8.67	0.57	0.96	0.68
VT	8.16	0.64	0.95	0.83
QA	6.29	0.53	0.99	<0.10
QE	10.04	1.15	0.97	0.33
QL	7.03	0.44	0.99	<0.10
QH	7.88	0.37	0.97	0.42
QT	5.73	0.73	0.95	0.86

3.3. Calorimetric measurements

The microcalorimetric results for all soils are listed in Table 5. The thermal effects can be used to distinguish the soils with the different additives. The sequential series of values decreases in thermal effect for cattle manure: QE ~ RE, VE; for municipal refuse compost: QL, RL and VL; for earthworm casts: QH ~ VH, RH.

The lower values for trifluralin are observed in relation to the control of 20% of moisture content in the soil, yielding 0.15 ± 0.01 kJ g^{-1} (RA), 0.15 ± 0.01 kJ g^{-1} (VA) and 0.23 ± 0.01 kJ g^{-1} (QA). These values indicated a possible inhibitory effect of this dose of trifluralin on the activity of microbiota present in the soil, as was observed when the pesticide 2,4-dichlorophenoxyacetic acid was monitored in Brazilian soil [22].

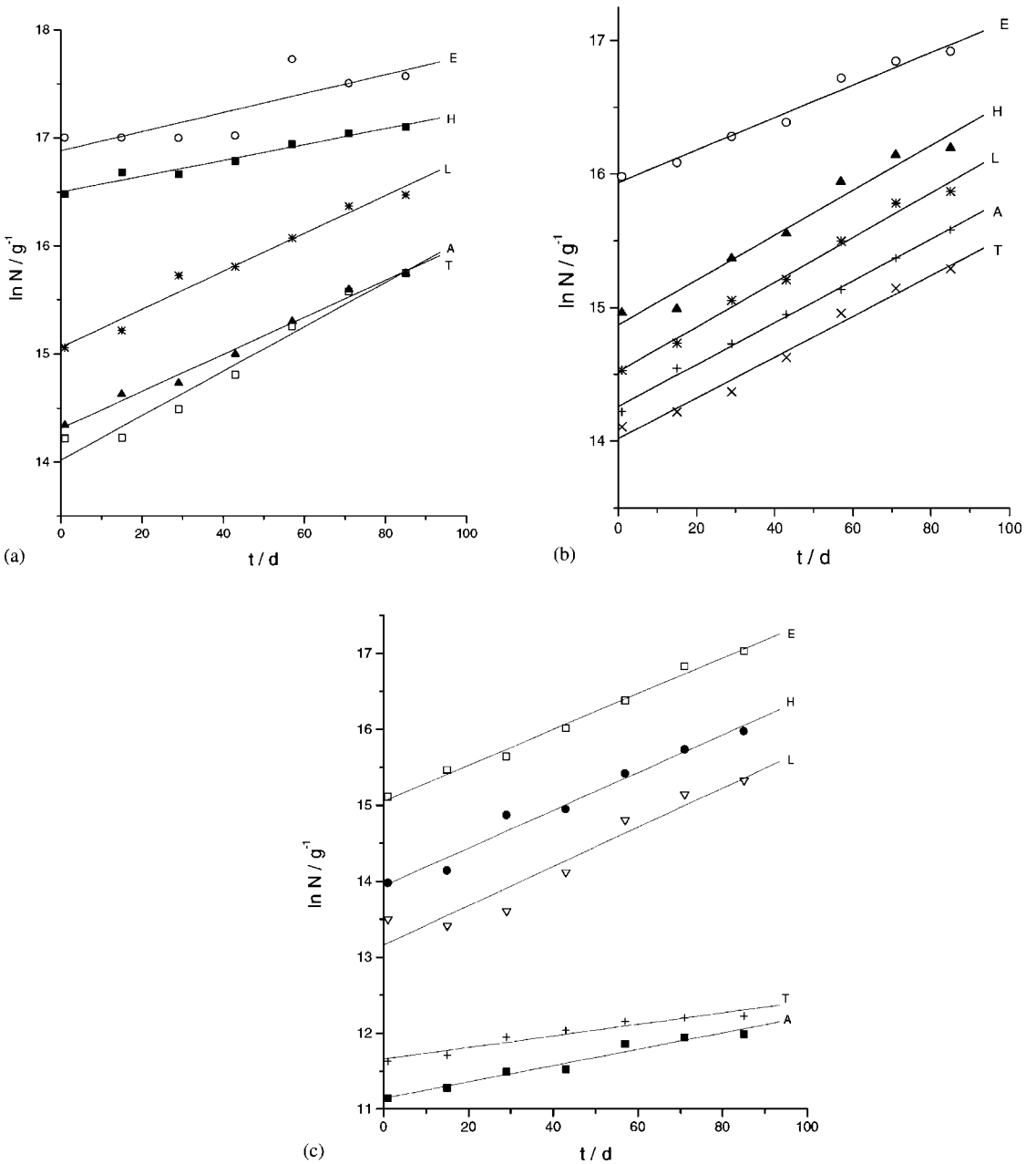

Fig. 1. The linear correlation between logarithm of bacteria number on dry soil samples of (a) Rhodic eutrudox, (b) Typic eutrudox and (c) Quartzipsamment, for (X = control (A), cattle manure (E), municipal refuse compost (L), earthworm casts (H) and trifluralin (T)) in incubation time (Inc) in days, cultivated in soil with extract medium at 301 ± 3 K.

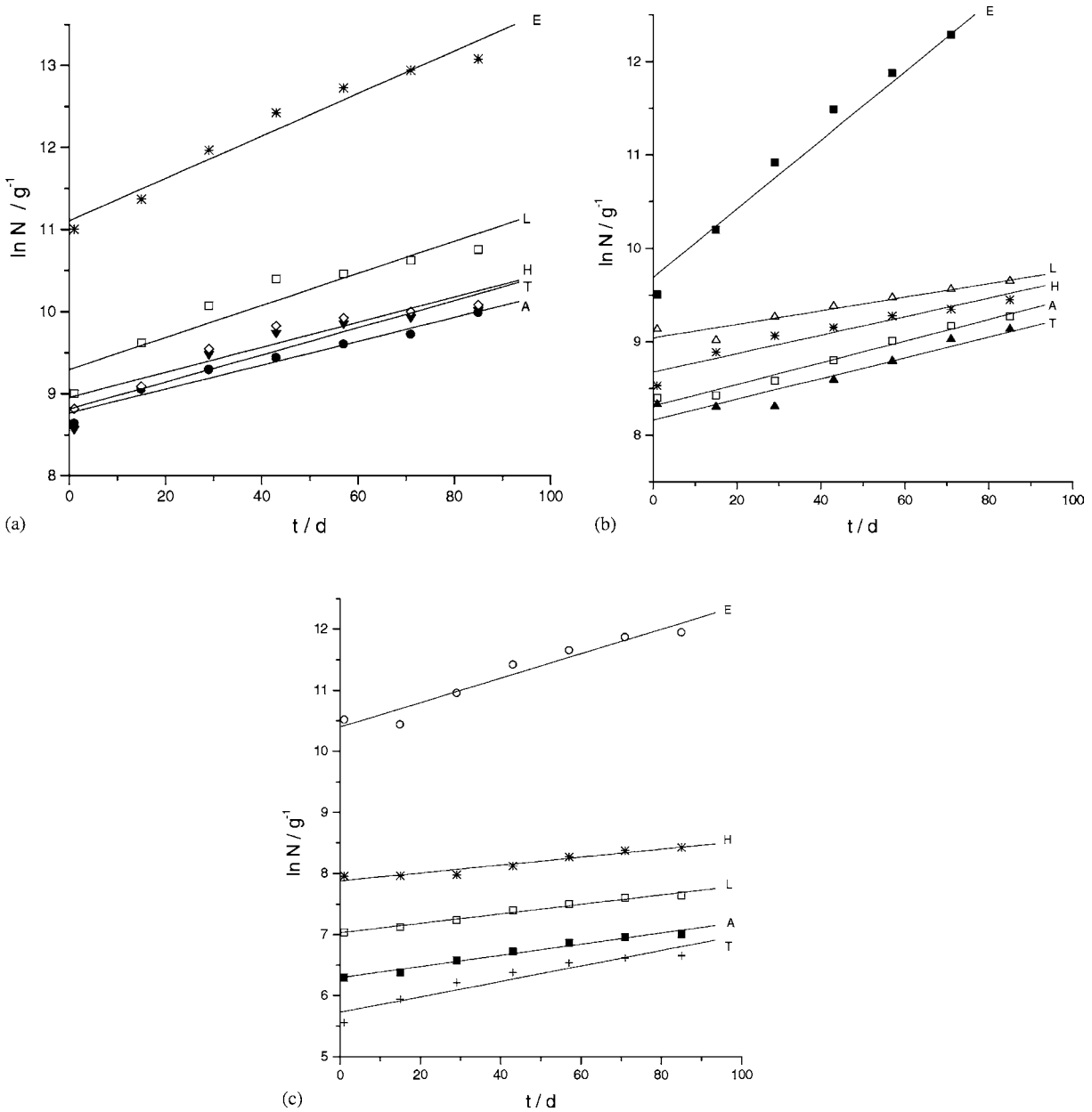

Fig. 2. The linear correlation between the fungi number on dry soil in soil samples of (a) Rhodic eutrudox, (b) Typic eutrudox and (c) Quartzipsamment, for (X = control (A), cattle manure (E), municipal refuse compost (L), earthworm casts (H) and trifluralin (T)) in incubation time (Inc) in days, cultivated in Martin medium, at 301 ± 3 K.

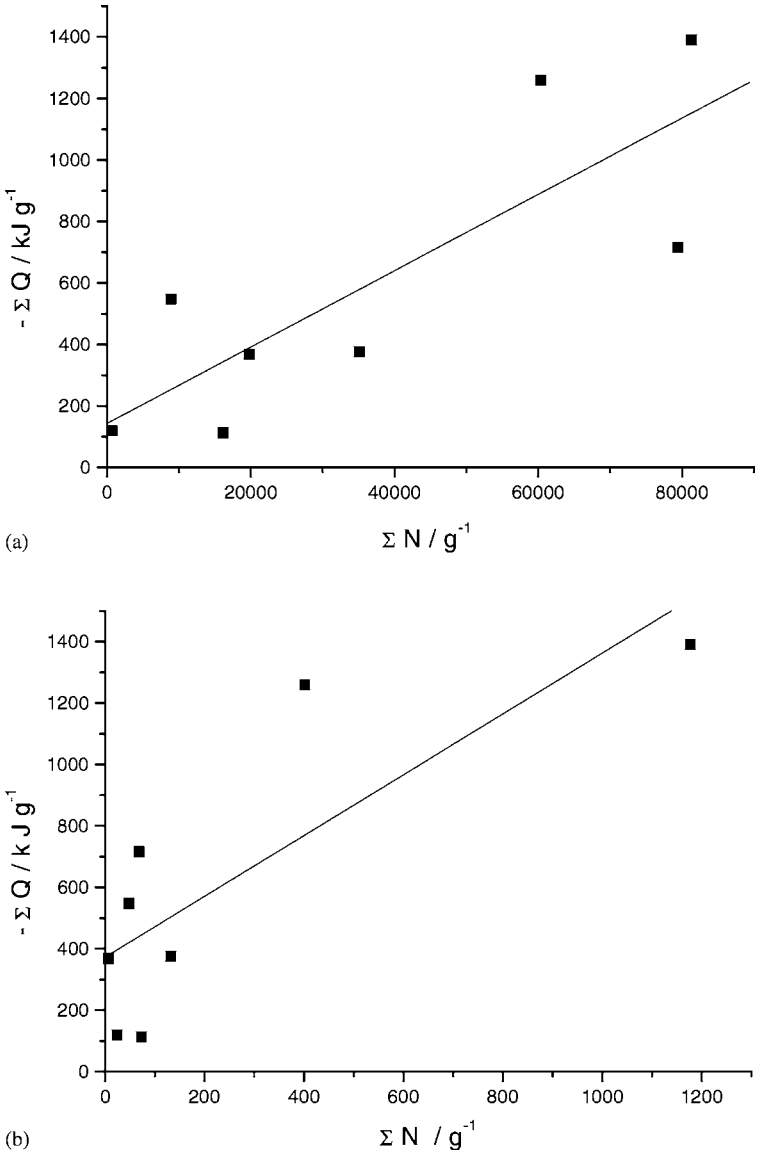

Fig. 3. Linear correlation between the sum of the thermal effects ($\sum Q$) as a function of the logarithm of the sum of the microorganisms ($\ln \sum N$) over 85 days of incubation time, for bacteria (a) and fungi (b).

3.4. Comparison of calorimetric method and microorganisms number

A linear correlation between the thermal effect obtained through calorimetry and bacteria and fungi number values is shown in Fig. 3(a) and (b), respectively. A lower correlation was obtained by considering the lesser incubation time for both bacteria and fungi. A careful interpretation in this case is necessary, because the number calculated from colony counts, might be different from the number of microorganisms that were presented or acting in the original sample, whose operations were monitored by calorimetry. This fact is due to the plate count operation which does not distinguish between active and dormant bacteria. If an inactive bacterial or fungal propagule is

Table 5
Results obtained from area (A_r) of power (ΔP) vs. time curve[a]

Soil	ΔP (μW)	A_r (cm^2)	$\sum Q$ (kJ g^{-1})
RE	600 ± 12	73.49 ± 1.47	1.286 ± 0.064
RL	600 ± 12	34.09 ± 0.68	0.644 ± 0.032
RH	700 ± 14	35.81 ± 0.72	0.772 ± 0.038
RT	35 ± 1	61.22 ± 1.22	0.066 ± 0.003
VE	800 ± 16	48.24 ± 0.96	1.107 ± 0.055
VL	500 ± 10	41.17 ± 0.82	0.610 ± 0.031
VH	650 ± 13	55.35 ± 1.11	1.041 ± 0.021
VT	65 ± 1	56.18 ± 1.12	0.098 ± 0.002
QE	800 ± 16	62.71 ± 1.25	1.352 ± 0.027
QL	850 ± 17	31.14 ± 0.62	0.713 ± 0.014
QH	600 ± 12	63.70 ± 1.27	1.030 ± 0.021
QT	140 ± 28	10.67 ± 0.21	0.040 ± 0.001

[a] The thermal effects ($\sum Q$) were recorded from the calorimetric curve with 1.50 g samples of soil containing 20% of moisture for Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q). The samples were charged with 25% of cattle manure (E) or municipal refuse compost (L) or earthworm casts (H) or 23 μg of trifluralin (T) for 103 days of incubation time, at 298.15 ± 0.02 K.

spread on a culture medium it will germinate and produce a colony that will be counted. However, the inactive propagule in the calorimetric system is not measured by this method [23].

This investigation clearly demonstrates that the methods measure different properties of the soil microbiota. The growth of particular communities of bacteria and fungi has less contribution than the total interactive effect of active microbiota in soil. The agar plate counts reflect a specific growth of bacteria and fungi cells, while the calorimetric method measures the total interaction of all microorganism growth in the system. However, the thermal effect reflects the competitive microenvironment in the soil and also other characteristics of the process, like enzymatic effects and the thermal degradation of compounds required for growth.

An increase in linear correlation for 85 days of incubation time for the two methods, yielding $r = 0.8181$ and $P = 0.0131$ for bacteria and $r = 0.8134$ and $P = 0.0140$ for fungi, were determined [16]. These tropical soils were previously investigated and a linear correlation between thermal effect and carbon dioxide evolution was detected [16,24]. A comparison between respirometry and calorimetry has been established in soils [25,26]. Over such incubation times, the specific bacteria and fungi communities in soil have major contributions in the thermal effect measured in this process. It is indicated that at the final incubation time, the soils have different conditions from the initial ones and establish a new equilibrium. In this phase, it is probable that other factor on influence of the microbial activity, may decrease in terms of importance in the global process, such as the competition of microorganism communities and the inhibitory effects of metabolites on dominant communities. In this final process, it is expected that a great part of the predominance of the labile organic compounds was degraded and the soil microenvironment can have specific different initial conditions. These conditions can be favorable for the development of adapted communities and other factors, associated with other communities, cause a drastic reduction in the initial population in the soil.

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