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Microcalorimetric measurements of the metabolic activity by bacteria and fungi in some Brazilian soils amended with different organic matter

Silvana A.M. Critter [a], Sueli S. Freitas [a,*], Claudio Airoldi [b]

[a] *Instituto Agronômico, Caixa Postal 28, 13001-970 Campinas, São Paulo, Brazil*
[b] *Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, 13084-971 Campinas, São Paulo, Brazil*

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Abstract

The effect of bacterial and fungal activities on organic matter degradation in Brazilian soils was studied by a microcalorimetric method. Bacteria and fungi isolated from tropical soils and added to: Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) soils amended separately with moisture (control) (A) and 25% of cattle manure (E), municipal refuse compost (L), earthworm casts (H) or 23 μg of trifluralin (T) were investigated. The number of colony forming units in soil suspension was quantified by microscopy and inoculated in respective soil. All processes were measured at intervals of 7 days over a period of 35 days. The exothermic thermal effect (μJ) per cm^3 of bacteria or fungi per gram of dry soil, respectively, for each substrate was: [(9 ± 1), (4 ± 1)] RA; [(478 ± 24), (105 ± 5)] RE; [(121 ± 6), (71 ± 4)] RL; [(121 ± 6), (71 ± 4)] RH; [(8 ± 1); (3 ± 1)] RT; [(10 ± 1), (9 ± 1] VA; [(347 ± 17), (261 ± 13)] VE; [(71 ± 4), (28 ± 1)] VL; [(22 ± 1), (33 ± 2)] VH; [(7 ± 1), (10 ± 1)] VT; [(19 ± 1), (12 ± 1)] QA; [(1301 ± 65), (46 ± 2)] QE; [(89 ± 4), (9 ± 1)] QL; [(130 ± 7), (11 ± 1)] QH; [(32 ± 2), (8 ± 1)] QT. The calorimetric values are higher for bacteria than for fungi. In general, the results showed higher activities in the soil amended with cattle manure than with other additives.

Keywords: Microcalorimetry; Brazilian soils; Fungi; Bacteria; Microbial activity

1. Introduction

The importance of soil microorganisms on the maintenance of soil fertility is well known [1]. Knowledge of organic matter (OM) turnover is relevant for understanding of carbon sequestration, nutrient cycling and biophysical attributes of soil within particular ecological and climatic regions [2].

Tropical soils have specific characteristics associated with their mineral fractions, and the climatic conditions also directly influence organic matter decomposition [3]. The contribution of microbial activity to organic material degradation is related to the quantities and availability of a labile carbon source. In this context, microorganisms also influence soil structure due to their role in organic matter decomposition and, consequently, on plant growth. Some researchers have reported that addition of a readily available substrate causes a rapid stimulation of soil microbiota [4].

The functional diversity of soil microbial communities includes a range of activities involved in many reactions, such as decomposition, nutrient transformation, plant growth promotion/suppression and various soil physical processes [5]. Fungi are involved in binding soil aggregates together with larger particles, whereas bacteria mainly influence stabilization of clay and silt particles [3]. Bacteria are found in diversity and numbers greater than other organism groups in natural soils and the products of their metabolism, like extracellular polysaccharides, also bind soil particles and contribute to soil structure. However, bacteria also attack the humic materials in organic matter/clay complexes and consequently affect the structure of soil aggregates [6].

Soil texture can be influenced by OM turnover. Clay and silt-sized particles can physically and chemically protect the OM fraction from decomposition due to isolation of compounds within and among soil microaggregates [2].

Studying microorganism behavior in soil is essential for a broader understanding of soil. Qualitative and quantitative changes in microbial communities may be used as indicators of short and long-term changes in soil. The activities of

* Corresponding author.
E-mail address: sfreitas@iac.sp.gov.br (S.S. Freitas).

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diverse fungal and bacterial communities are measured by assessing the variation of thermal effect [7].

The microbial activity in heterogeneous systems like soil has been assessed by calorimetric methods [8,9]. Calorimetry is an important tool for measuring metabolic activities of cells and biological tissues where the thermal effect is usually measured in a system under controlled temperature conditions. However, a disadvantage of calorimetry is the non-specificity of the method [8,10].

Investigations have also been carried out by following other determinations of metabolism, such as carbon dioxide evolution [8,11–13]. Therefore, it is possible to establish the soil microbial activity and characterize these cellular systems [14,15].

From an ecological point of view, soil is an important system to be explored with calorimetric methods. Previous results on tropical soils [16–20] are good illustrations of the practical usefulness and potential of isothermal calorimetry.

Such instrumentation has been applied to the study of diverse processes, such as the effects of environmental conditions on the growth of petroleum microbe strains, isolated from oil reservoirs [21], and to assess microbial growth parameters [22].

The aim of the present investigation is to obtain information about: (i) the quantitative contribution of microbial activity of isolates of bacteria and fungi of Brazilian soils amended with organic material (cattle manure, municipal refuse compost and earthworm casts) and with the agrochemical trifluralin and (ii) the influence of organic material on the activity of isolates in soils with different textures. The organic materials to be amended with soils were chosen due to their great application in agricultural systems and also because reflect some diversity of substrates found in the environment. The microbial activity of the isolated bacteria and also fungi was monitored by a heat conduction calorimeter. All values were obtained at intervals of 7 days, over a period of 35 days incubation.

2. Experimental

2.1. Soils and sampling

The soils were selected to obtain sandy, sandy/clay medium and clay textural classes. Brazilian soil samples Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) soil [23,24], were collected at a depth of 5–10 cm, after removal of litter in the surface layer. These samples were air dried, homogenized by sieving to less than 2 mm to separate roots and large particles, and stored at 293 ± 3 K [25,26].

2.2. Analytical methods of chemical characterization of soil

Moisture content was determined by drying the soil sample to a constant mass. Organic matter (OM) of each soil sample was determined in an acidic medium, with the end point followed by a redox reaction [24–26]. 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 ($H^+ + Al^{3+}$) was obtained 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 percolating a solution of 5.0×10^{-2} mol dm^{-3} nitric acid through a column containing 10.0 g of the soil with 0.10 dm^3 of solution. Na^+ and K^+ were analyzed by flame photometry using a previously obtained calibration curve. The cations Mg^{2+}, Ca^{2+} and Al^{3+} were determined through atomic absorption spectrometry [27,28].

The water holding capacity of the soil was measured in tubes with a known relationship between the amount of soil and volume of water, for calculating 60% of the maximum water holding capacity [25].

2.3. Microorganism purification and isolation

Bacteria and fungi colonies were isolated from suspensions of soil samples (R, V or Q) with 7.50 g of soil amended with 2.50 g of organic material: cattle manure (E), municipal refuse compost (L), earthworm casts (H) or 23 μg of trifluralin (2,2,2-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) (T), giving an equivalent dose of agrochemical of 1.25 kg ha^{-1}. After addition of 90.0 cm^3 of sterilized distilled water to the 10.0 g sample, the flasks were closed and the contents were then stirred for 30 min. From this suspension, 1.0 cm^3 was inoculated in plates with Martin medium for fungi and soil extract medium for bacteria and incubated for two days at 301 ± 3 K [29]. The organisms were isolated in base on their macroscopic appearance, size, and predominant colonies, quantities and proximity. It is observed in the dish plates during the isolation process, with successive passages in dish plates to confirm purification of the isolate. The initial organism number must be standardized because it can affect the area under the power-time curve.

To obtain a suspension to inoculate sterilized soil samples, a volume of 10.0 cm^3 of sterilized water was added to dish plates where isolates of bacteria and fungi were growing exponentially [30].

The number of cells in suspension was directly counted by microscope, using Neubauer slides, to obtain the number of cells contained in the suspension volume [30].

Areas of 1.0, 0.0625 or 0.0025 mm^2 with respective volumes of 1.0×10^{-4}, 62.5×10^{-7} or 2.5×10^{-7} cm^3, were chosen. Suspensions were homogenized, transferred to the Neubauer, and counting was performed with a Carl Zeiss microscopic (Jenaval) in five different aliquots of the suspension [30]. The suspension was adjusted by dilution to reach the 150–200 cellules mm^{-1} range with ±5% uncertainty.

2.4. Microcalorimetry

The thermal effect in samples was measured in an LKB 2277 isothermal calorimeter, manufactured by Thermometric. It is a twin, differential heat conduction microcalorimeter operating in the static mode and measures the heat flow rate. In the condition that the temperature of the sample is higher than the temperature of the surroundings, heat flows from the sample to the surroundings, keeping the temperature of the sample constant. In this system, the measurements are due the changes in the flow of heat, and are proportional to the metabolic rates of the organisms. The calorimeter was calibrated by the release of electrical energy in a resistor of the instrument [20].

The measurement was done with 5.0 cm^3 stainless steel ampoules. Teflon sealing discs were used to prevent evaporation and transfer of oxygen and carbon dioxide in the hermetically closed ampoules, with experiments carried out at 298.15 ± 0.02 K. All determinations were performed in ampoules autoclaved for 30 min at 393 K with 0.75 g of soil plus 0.25 g of organic material as described above. To this sterilized soil sample was added 0.25 cm^3 of bacteria or fungi aqueous suspension. An identical ampoule containing 1.0 cm^3 of distilled water was used as reference. In the case of trifluralin, 23 μg of this compound was transferred to the aqueous solution while the control was formed by 0.75 g of soil plus 0.25 g of organic material with 0.25 cm^3 of distilled water and autoclaved for 30 min at 393 K. The sequential time of incubation and measurements were: 1, 7, 14, 21, 28 and 35 incubation days and air was admitted to ampoules between measurements. The ampoules were stored between measurements in a special room with the temperature controlled at 298 ± 3 K [20]. Each isolate was inoculated in sterilized samples of the same combination amendment-soil that it was obtained from.

The thermal effect was recorded after 1 h of stabilization of the base line for each measurement. The obtained values were plotted as a function of time and the results were calculated by integrating the area of the power versus time curves. All thermal-effect results were obtained from duplicate runs [9].

A parallel sequence of experiments with 7.50 g of soil plus 2.50 g of additive was performed for each amendment for pH measurement at the initial time of sampling.

2.5. Statistical analyses

All values are based on soil samples dried in an oven. All results are given as an arithmetic mean for two independent determinations for each incubation time. The standard percent error was calculated for each amended soil for each incubation time used. The ANOVA method was used for statistical analysis with as significance, $P \leq 0.05$ level of difference, between means with correction factors, including amended soil and incubation time. However, the analyses resulted in a statistical parameter lower than the chosen value.

3. Results and discussion

3.1. Soil characteristics

A summary of the main properties of the soils and the organic compounds are given in Table 1. R and V soils showed the highest P, OM and CEC values, as opposed to Q soil. The three soils are acidic in nature and the SB values are very low, medium and high for Q, R and V soils, respectively. All the additives, E, H and L, had higher values of OM and nutrient contents, while the pH is near neutral. The same characteristic is also observed for SB percentage and CEC values.

3.2. Microcalorimetry

As an example, typical calorimetric curves for bacteria and fungi are shown in Fig. 1. The straight line connecting the initial and final points is used as a baseline. The positive baseline indicates that activity began before the initial measurements. However, all measurements were standardized and measured 1 h after inoculation in aqueous suspension of the organisms.

Table 1
Main chemical properties of soils and organic materials: organic matter (OM), pH, phosphorus (P), exchangeable cations (K, Ca, Mg), potential acidity (H + Al), sum of 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)

Property/soil	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 (mmol dm^{-3})	1.0 ± 0.1	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

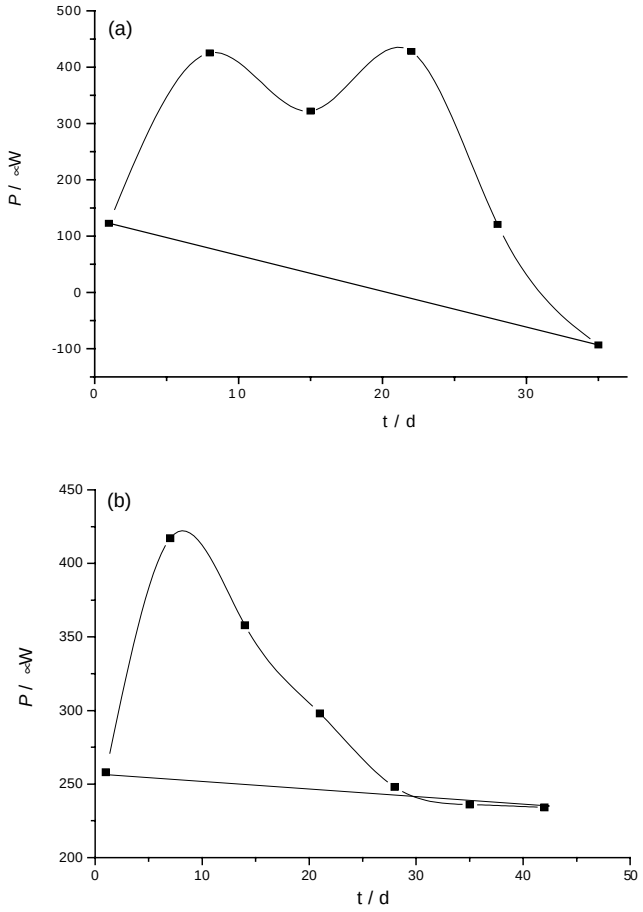

Fig. 1. Calorimetric power time curves obtained for Rhodic eutrudox soil amended with cattle manure (RE) and inoculated with bacterial (a) and fungal (b) isolates at 298.15 ± 0.02 K.

The exothermic thermal effect (Q_1), expressed in joules per gram of dry soil, was calculated from the area (Ar, cm^2), resulting of the curve power (ΔP, μW) versus time (t, day). The exothermic net thermal effect (Q_{net}), was calculated in joules per gram of dry soil (Q_1) per number of organisms (n_1) contained in 0.25 cm^3 of suspension, where: $n_1 = n \times 0.25\,cm^3$.

All results obtained are listed in Table 2 for bacteria and Table 3 for fungi. The calorimetric curves showed one or two peak times (PT). The area values were different for the majority of investigated systems.

The microbial calorimetric curve after 35 days of incubation time showed one or two peak times, as shown in Fig. 1. When there were two PT, the one marked with a superscript indicates the maximum peak. These results suggest that the observed peaks are related to two plateaux of activities for the degradation of organic materials. In the first stage a degradation of organic compounds takes place and the products of these reactions are used by the same species during the second peak.

The net exothermic thermal effect (Q_{net}) for the calorimetric curves in Tables 2 and 3 reflect the sequential increase in the activity for bacteria: VT ∼ RT ∼ RA ∼ VA < QA < QT < VL ∼ QL < RL ∼ RH ∼ QH ∼ VH < VE < RE < QE. The thermal effect produced by the action of bacteria in the soil amended with cattle manure presented values higher than those of the earthworm casts (H) and municipal refuses compost (L). This sequence shows that the bacteria species isolated from soil amended with cattle manure have larger values in sandy soil (Q) than in other clay soils (R or V). This result is adverse in relation to soil characteristics, where the natural soil sandy (Q) has the lowest values of OM, pH, SB and CEC, as shown in Table 1. This result

Table 2
Power variation (ΔP) as a function of incubation time over 35 days, pH, peak time (PT), area (Ar) for power time curve, thermal effect (Q_1), number of organisms (n) per 1.0 cm^3 of suspension and net thermal effect (Q_{net}) for isolated bacteria in Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) soils containing 20% water: the control (A) and 25% of cattle manure (E), municipal refuse compost (L), earthworm casts (H) or 23 μg trifluralin (T) at 298.15 ± 0.02 K

Soil	pH	PT (day)	ΔP (μW)	Ar (cm^2)	$-Q_1$ ($J\,g^{-1}$)	n ($\times 10^6/cm^{-3}$)	$-Q_{net}$ (μJ $org^{-1}\,g^{-1}$)
RA	5.0 ± 0.3	7/20	350 ± 17.5	62 ± 3	273 ± 14	129 ± 6	9 ± 1
RE	6.4 ± 0.3	7/21[a]	550 ± 27.5	90 ± 4	746 ± 37	7 ± 1	478 ± 24
RL	5.2 ± 0.3	10[a]/28	600 ± 30	76 ± 4	571 ± 29	19 ± 1	121 ± 6
RH	7.0 ± 0.4	8[a]/21	900 ± 45	78 ± 4	889 ± 44	29 ± 2	121 ± 6
RT	5.0 ± 0.3	22	610 ± 31	26 ± 1	71 ± 4	36 ± 2	8 ± 1
VA	6.2 ± 0.3	13[a]/29	120 ± 6	53 ± 3	85 ± 4	34 ± 2	10 ± 1
VE	6.9 ± 0.3	10	1000 ± 50	69 ± 4	1061 ± 53	12 ± 1	347 ± 17
VL	6.3 ± 0.3	20	400 ± 20	60 ± 3	376 ± 19	21 ± 1	71 ± 4
VH	6.9 ± 0.3	8	800 ± 40	49 ± 3	577 ± 29	114 ± 6	22 ± 1
VT	6.2 ± 0.3	16	70 ± 35	37 ± 2	154 ± 8	49 ± 2	7 ± 1
QA	4.7 ± 0.2	7/29	25 ± 1	71 ± 4	26 ± 1	8 ± 1	19 ± 1
QE	7.1 ± 0.4	8	1000 ± 50	28 ± 1	433 ± 22	3 ± 1	1301 ± 65
QL	5.1 ± 0.3	18	300 ± 15	23 ± 1	89 ± 4	6 ± 1	89 ± 4
QH	7.0 ± 0.4	8[a]/21	350 ± 18	25 ± 1	151 ± 8	11 ± 1	130 ± 7
QT	4.7 ± 0.2	15[a]/28	50 ± 3	41 ± 2	34 ± 2	8 ± 1	32 ± 2

[a] The most intense peak time.

Table 3
Power variation (ΔP) as a function of the incubation time over 35 days, pH, peak time (PT), area (Ar) for power time curve, thermal effect (Q_1), organisms number (n) per 1.0 cm^3 of suspension and net thermal effect (Q_{net}) for isolated fungi in Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) with 20% water: control (A) and 25% of cattle manure (E), municipal refuse compost (L), earthworm casts (H) or 23 μg trifluralin added (T) at 298.15 ± 0.02 K.

Soil	pH	PT (day)	ΔP (μW)	Ar (cm^2)	$-Q_1$ ($J\,g^{-1}$)	n ($\times 10^6/cm^{-3}$)	$-Q_{net}$ (μJ $org^{-1}\,g^{-1}$)
RA	5.0 ± 0.3	20[a]/35	150 ± 8	40 ± 2	92 ± 5	85 ± 4	4 ± 1
RE	6.4 ± 0.3	7	250 ± 13	52 ± 3	203 ± 11	78 ± 4	105 ± 5
RL	5.2 ± 0.3	10/28[a]	45 ± 2	50 ± 3	50 ± 3	3 ± 1	60 ± 3
RH	7.0 ± 0.4	21	220 ± 11	48 ± 2	154 ± 8	9 ± 1	71 ± 4
RT	5.0 ± 0.3	21	450 ± 23	4 ± 1	29 ± 1	44 ± 2	3 ± 1
VA	6.2 ± 0.3	28	180 ± 9	26 ± 1	58 ± 3	25 ± 1	9 ± 1
VE	6.9 ± 0.3	10	600 ± 30	69 ± 3	523 ± 26	78 ± 4	267 ± 13
VL	6.3 ± 0.3	10[a]/31	275 ± 14	50 ± 3	177 ± 9	26 ± 1	28 ± 1
VH	6.9 ± 0.3	15	800 ± 40	49 ± 2	497 ± 25	61 ± 3	33 ± 2
VT	6.2 ± 0.3	22	100 ± 5	37 ± 2	47 ± 2	19 ± 9	10 ± 1
QA	4.7 ± 0.2	21/34[a]	450 ± 23	197 ± 10	1288 ± 64	421 ± 21	12 ± 1
QE	7.1 ± 0.4	22	750 ± 38	27 ± 1	310 ± 16	27 ± 1	46 ± 2
QL	5.1 ± 0.3	28	275 ± 14	23 ± 1	82 ± 4	35 ± 2	9 ± 1
QH	7.0 ± 0.4	14/29[a]	170 ± 9	25 ± 1	55 ± 3	20 ± 1	11 ± 1
QT	4.7 ± 0.2	22	750 ± 38	41 ± 2	385 ± 19	191 ± 10	8 ± 1

[a] The most intense peak time.

is attributed to characteristics of organic material amended where the highest availability of labile organic compounds, phosphorous and bases present in the cattle manure, which was favorable for a stimulation of the microbial activities, in spite of such soil presented lower quantities of organic matter, nutrient and acidic conditions [31]. Thus, the addition of organic material altered many conditions for bacteria species development, including the contribution of pH of medium after addition of organic materials, as shown in Tables 2 and 3. On the other hand, no differentiation was observed for the results involving isolated organisms of soil with earthworm casts and in this case the characteristics of soil does not show effect on bacterial activity of this specific species.

The sequence for increasing of activity for fungi, in Table 3 is: VT ∼ RA ∼ QA ∼ VA ∼ QH < RH ∼ QT ∼ QL ∼ RT < VL ∼ VH < QE < RL < RE < VE. The thermal effect is higher for VE than for other additives. This result for fungal species is in agreement with the soil characteristics, where the clays soils (V and R) with the highest quantities of OM, CTC and SB, showed also the highest activities for the fungal for each amended studied. But the above sequence shows that this fungal activity is not ideal for characteristics of organic material, which can show for RL with high values than QE. As observed, the sequence of fungi activity observed was different from bacteria.

Thermal effect versus incubation time is shown in Fig. 2 for bacteria (a) and fungi (b). These figures show the thermal effects for bacteria are higher than those for fungi. Bacteria apparently have an advantage over fungi in use of these substrates. The results also show variable efficiency of substrate use among the different isolated species. So, different species present distinct capacities for organic material degradation.

The bacteria and fungi isolated from cattle manure, municipal refuse compost and humic material have a larger activity in the degradation of organic materials when compared with the natural control soil, as shown in Fig. 2(a) and (b). The great difference in comparison to the control soils suggests too that the metabolic activity of microorganism in these soils can be negligible. This result is directly related with the low effective substrate concentration in comparison to amended soils. However, such soils are in agreement with an existence of a favorable correlation between the addition of organic matter content and the microbial activity.

For the bacteria groups shown in Fig. 2(a), taking into account the same kind of soil, such as R, V or Q, a differentiation in the activity among of the different types of organic material, for example, VE, VH, VL, VT and control was observed, for all treatments, with exception of RL and RH. If the same organic material for different soils are compared, then only a differentiation for the cattle manure was detected, as well as a difference between VL in relation to RL ∼ QL. However, for humic material no distinction was observed (RH ∼ VH ∼ QH). The control soils, RA, VA and QA, have the same microbial activity in relation to trifluralin. This implies that 23 μg of this agrochemical per gram of soil has a non-inhibitory effect for bacteria. On the other hand, the data indicate that cattle manure promotes a higher microbial activity, resulting in a better differentiation among the values.

By comparison among the fungi groups, as presented in Fig. 2(b), differentiation among the same soil with distinct amending materials, for example, RE, RL, RH, and RT, was observed and also among different soils amended with the same organic material, for example, VE > RE > QE. However, trifluralin showed large activity in relation to the control for fungi. It is possible that fungi were able to use trifluralin as a source of energy, differently from bacteria.

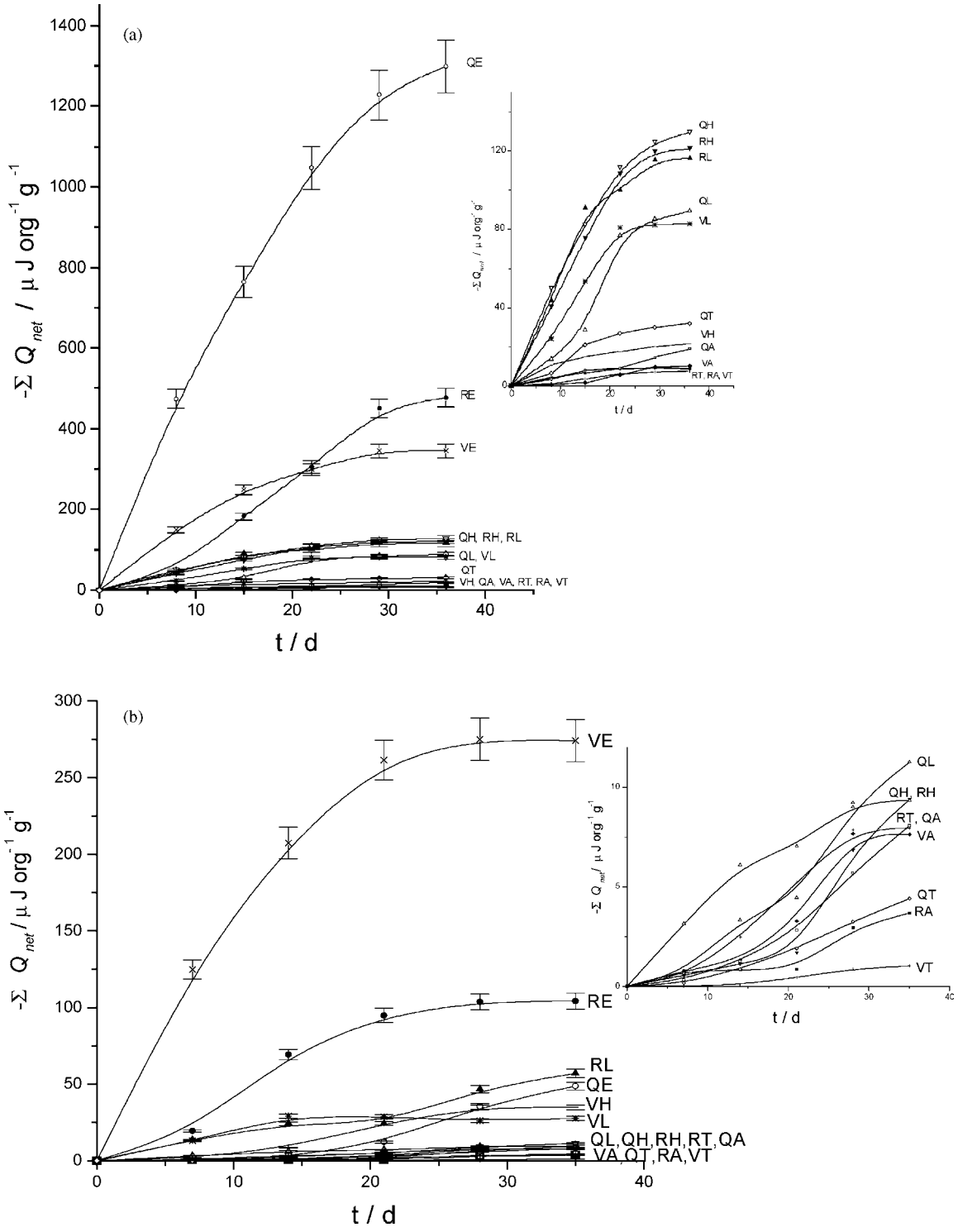

Fig. 2. Thermal effect (Q_1) from microbial degradation of bacterial (a) and fungal (b) isolates when inoculated in soils: Rhodic eutrudox (R), Typic eutrudox (V) and Quartzipsamment (Q) with 20% of water, control (A) and amended with 25% of cattle manure (E), municipal refuse compost (L), earthworm casts (H) or 23 μg trifluralin (T) at 298.15 ± 0.02 K.

4. Conclusion

The calorimetric method showed contributions for the evaluation and differentiation of the microbial activity of different communities on Brazilian soils, even though the majority of the techniques normally used in this field are more specific.

The results illustrate that the soil structure influences the microbial activity and that bacteria and fungi activities were favored on cattle manure. This organic material is considered

as a treatment of high quality, due the large content of organic matter and nutrients, which are fundamental elements for microorganism metabolism. The importance of organic matter has been well established due its favorable influence in formation and stabilization of soil aggregates, stimulating of the microbial activity and cycling of nutrients.

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