ELSEVIER

Thermochimica Acta 394 (2002) 171–178

thermochimica acta

www.elsevier.com/locate/tca

Effect of deforestation on soil microbial activity A worm-composite can improve quality? A microcalorimetric analysis at 25 °C

E. Elizabeth Sigstad*, Maricel A. Bejas, M. Julia Amoroso, Celina I. García

Institutos de Química Orgánica y de Microbiología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, CP T 4000 INI, Tucumán, Argentina

Received 10 September 2001; received in revised form 12 February 2002; accepted 5 March 2002

Abstract

All crops grow in the soil environment; thus, soil properties are of great importance to determine plant and crop conditions. An index to assess soil fertility is its microbial activity. In this sense, in the present work we report the microbial activity of three soils selected by age of deforestation as determined by microcalorimetry at 25 °C and its correlation with organic matter content and colony formation units in two growth media. Also the effect of a worm-composite, currently used as soil fertiliser, on the poorest soil is evaluated.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Isothermal microcalorimetry; Soil quality; Microbial activity

1. Introduction

All crops grow in the soil environment; thus, soil properties are of great importance to determine plant and crop conditions. Nowadays, arable land is becoming progressively deteriorated due to bad habits of soil and crop handling such as excessive deforestation, lack of crop rotation, wrong practices of irrigation and excessive pesticide applications. As a consequence, productivity decreases.

Tucumán Province, Argentina, has an economy mainly based on agriculture. Eastern semiarid areas have been completely deforested. A common practice is the production of soybean, wheat, sugarcane, lemon and tobacco. Sub-tropical areas in the West have been traditionally harvested with sugarcane but lemon trees and tobacco progressively replace the old plantations. A common practice during sugarcane harvest since many years ago was to burn it. Also, fumigation with several biocides and irrigation with contaminated waters is very frequent. All these practices are causes of soil deterioration.

An index to assess soil fertility is its microbial activity [1]. Soil microbial community is highly heterogeneous. Among them, bacteria are the most and it is through their metabolic activity that minerals and soil organic matter are transformed in a way that important nutrients such as N, P, and S are simultaneously converted into assimilable forms for plant and other micro-organisms. As a result, these micro-organisms

* Corresponding author. Present address: Paraguay 668, CP T 4107 DDN, Yerba Buena, Tucumán, Argentina.
Fax: +54-381-248169.
E-mail addresses: esigstad@arnet.com.ar, lizzie@unt.edu.ar (E.E. Sigstad).

0040-6031/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0040-6031(02)00267-8

play an important role in the decomposition of organic matter, nutrients recycling and soil formation.

Most of the soil micro-organisms are viable but not cultivable. Microcalorimetry allows determination in situ, therefore is more reliable than routine microbiological methods. Many authors have used calorimetry for measurements of soil microbial activity [2-7 and Ref. in 8].

In this work, we report the microbial activity of three soils selected by age of deforestation as determined by microcalorimetry at 25 °C and its correlation with organic matter content and colony formation units in two growth media. Also, the effect of a worm-composite used as soil fertiliser on the poorer soil is evaluated.

2. Experimental

2.1. Soil sampling

Soil samples were collected on May 1999 from three soybean fields from the southeast area of Tucumán Province, Argentina, according to the age of deforestation ((A) 3 years; (B) 6 years and (C) 15 years). Ten sites were randomly chosen on each field and sub-samples were collected up to a depth of 15 cm after removing the very top layer. After combination of sub-samples they were sieved ($2 \times 2\,mm^2$) to remove rests of roots and coarse material. An aliquot was taken to determine soil sampling humidity and the remaining sample was air-dried over a week and further stored in polyethylene bags at 5 °C until used (3 months). This treatment allows exchange of O_2 and CO_2 but not water vapour and ensures reproducibility of calorimetric results [5].

2.2. Physicochemical determinations

Moisture content (MC) was determined by drying an aliquot (×2) until constant weight at 105 °C [9]. Field capacity humidity (FCH) was determined by preparing a glass column, of the column chromatography type (20 mm i.d.) with soil (450 mm) and left to equilibrate over a baker containing distilled water during a week. Then, moisture content of samples taken 50–100 mm above the water level was determined [5]. pH was measured with a glass electrode on a suspension of soil in deionised water (1:2.5) [9]. Organic matter (OM) was determined by oxidation with $K_2Cr_2O_7/H_2SO_4$ [9].

2.3. Microbiological determinations

Colony formation units per gram of dry soil ($CFU\,g^{-1}$) were determined by the plate dilution method in two different growth media (agar-agar and Trypteine Soy Agar (TSA)). The plates were incubated at 25 °C over a week or two [2,10].

2.4. Inoculation with worm-composite (Nutrilomb-20; UNRC)

Nutrilomb-20 is a composite made with the excrements of worms (*Eisenia foetida*) and further work-up with yeast and other micro-organisms. The inoculation process was performed by placing soil samples with an MC of $0.15\,g\,g^{-1}$ (about 110.0 g dry weight) in a beaker (75 mm i.d × 75 mm). The worm-composite was diluted to a 5% solution with sterile water and 0.4 ml of this solution was used to inoculate the soil samples as follows: C_1, inoculated with nutrilomb; C_2, inoculated with sterile (membrane filtration, 0.2 μm) nutrilomb; C_3, control of non-sterilised samples; C_4, control for soil samples C_5 and C_6 treated at 121 °C, 1 atm during 20 min; C_5, inoculated with nutrilomb and C_6, inoculated with sterile nutrilomb. Samples were left at laboratory room temperature during January and February (35–40 °C) and then, air-dried and stored as stated previously until used.

2.5. Calorimetric determinations

A twin heat conduction type microcalorimeter (Lund University, Sweden) as described elsewhere [11] was used. Soil sample (1000.0 ± 5.0 mg) equilibrated at the desired MC in polyethylene bags during 24 h was evenly placed on the bottom of the calorimetric ampoule ($8.0\,cm^3$) and a glucose solution (0.05 ml) was added to record the thermal power (P)–time (t) curve of microbial growth. Control experiments were performed with 0.05 ml sterile water. By using the Origin 4.0 program (Microcal, Inc.), the curves obtained were processed.

Results reported are the mean of at least three replicates (±S.D.) and referred to soil dry weight.

3. Results and discussion

Table 1 shows the physicochemical and microbiological properties for soils A (3 years), B (6 years) and C (15 years) since deforestation and soil A with a moisture content of: (a) 5% and (a′) 13%. It is interesting to note that CFU g^{-1} values for soil A with 4.11 and soil B with 3.17% OM are higher in TSA whereas the highest value for soil C with 1.88% OM is in agar-agar. TSA is a rich growth media whereas agar-agar is very poor. Microbial growth in the soil occurs under conditions where a mixture of populations with different affinities for the substrate coexists. Soil C is microbiologically and chemically very poor and the populations that prevail in it need less concentration of nutrients. Thus, these populations need a longer time to be observed in a growth medium and therefore a poor one is more convenient; their metabolism is different from that of micro-organisms used to a rich environment. These higher CFU g^{-1} values are linearly related to OM ($g\,g^{-1}$) values through the following equation:

$$\mathrm{CFU}\,g^{-1} = 1.61 \times 10^7 + 22.7 \times 10^7 \times \frac{\mathrm{OM}}{100},\quad r^2 = 0.99 \qquad (1)$$

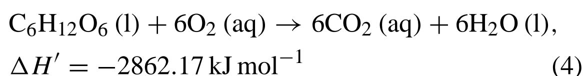
On the other hand, when soil is stored under stress conditions (water stress) as for samples (a) and (a′) micro-organisms need some time to adapt on the addition of water. When a dilution of the soil suspension in water is spread on a poor media as agar-agar and left to incubate until maximal growth, most of the micro-organisms have time to grow. Counting in this case might include fungi and other micro-organisms in addition to bacteria whereas in soil stored under optimal MC (i.e. soils A–C) the micro-organisms that grow are those more adapted consuming all nutrients of the sample. The CFU g^{-1} values obtained in agar-agar for soil A with different MC are related to the respective MC through the following equation:

$$\mathrm{CFU}\,g^{-1} = 1.31 \times 10^8 – 4.04 \times 10^8 \times \frac{\mathrm{MC}}{100},\quad r^2 = 0.99 \qquad (2)$$

Fig. 1 shows average specific thermal power (p)–time (t) curves due to the degradation of 4.0 mg glucose g^{-1} dry soil by the micro-organisms contained in soil A with: (a) 5%, (a′) 13% and (A) 27% MC. Table 2 shows the thermodynamic parameters as determined from the curves in Fig. 1. Note that the growth peak with a peak time (t_{peak}) at 41 h for sample A at FCH. The intensity of this peak (p_t) decreases with decreasing MC of the samples. The peak at 8.5 h is more intense than the one at 41 h for soil samples (a) and (a′). At FCH water potential in the soil is about −0.03 MPa [1]. Micro-organisms are deeply affected by water potential. Some micro-organisms are tolerant to water stress but others are not and these latter ones might be responsible for the peak at 41 h. A linear relation was found between the metabolic heat, $\Delta H'_{\mathrm{met}}$, and the CFU g^{-1} values as determined in TSA as shown in the following equation:

$$\Delta H'_{\mathrm{met}} = -120.7 - 5.64 \times 10^{-5} \times \mathrm{CFU}\,g^{-1},\quad r^2 = 1.00 \qquad (3)$$

The glucose consumed (m_{cons}) was determined by referring the q_{obs} values to the heat of glucose oxidation [8] according to the following equation:



$$C_6H_{12}O_6\,(l) + 6O_2\,(aq) \rightarrow 6CO_2\,(aq) + 6H_2O\,(l),\quad \Delta H' = -2862.17\,\mathrm{kJ\,mol^{-1}} \qquad (4)$$

Table 1
MC of samples at the time of experiment, FCH, pH, OM and CFU g^{-1} values as determined by using agar-agar and TSA as growth media for soils A (2–3 years), B (5–6 years) and C (10–15 years) after deforestation and soil A with an MC of (a) 4.7% and (a′) 12.9%

	A	B	C	a	a′
FCH (%)	27.80 ± 0.20	22.40 ± 0.40	23.00 ± 0.30	27.80 ± 0.20	27.80 ± 0.20
MC (%)	26.60 ± 0.10	22.30 ± 0.10	22.40 ± 0.10	4.70 ± 0.20	12.90 ± 0.10
pH	7.25	6.92	6.51	7.25	7.25
OM (%)	4.11 ± 0.02	3.17 ± 0.08	1.88 ± 0.01	4.11 ± 0.02	4.11 ± 0.02
10^{-7} CFU g^{-1} agar-agar	2.29 ± 0.27	1.11 ± 0.16	2.09 ± 0.06	10.10 ± 1.10	8.00 ± 0.30
10^{-7} CFU g^{-1} TSA	2.53 ± 0.16	2.41 ± 0.25	1.07 ± 0.15	1.38 ± 0.10	1.44 ± 0.28

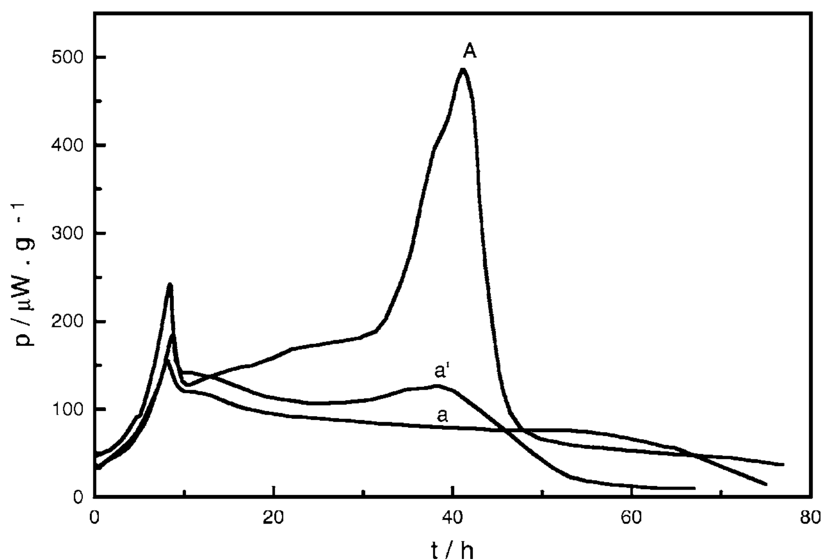


Fig. 1. Average specific thermal power–time curves for the degradation of 4.0 mg g^{-1} glucose by the microbial population of soil: (A) 26.6 (FCH), (a′) 12.9 and (a) 4.7% MC.

The fact that not all glucose was consumed is understandable if one considers that this substrate is not the best for soil micro-organisms and a nitrogen source was not added.

Experiments were also performed with soil A at FCH amended with 1.2 and 0.4 mg g^{-1} glucose. Results are shown in Fig. 2. It is interesting to note how the glucose concentration becomes limiting for micro-organisms involved with the peak at 41 h. A linear correlation was found between the q_{obs} and the amount of glucose added (m), as in the following equation:

$$q_{obs} = 3.36–9.313 \times \mathrm{m}, \quad r^2 = 0.99 \tag{5}$$

The calculated heat value of -9.313 J g^{-1} corresponds to $\Delta H'_{met} = -1677.8$ kJ mol^{-1} for the degradation of glucose. In view of these results, soils B and C were investigated at FCH. The intensity of the peak at 41 h decreased from soils A to C thus, with age of deforestation becoming almost imperceptible for soil C (not shown). Therefore, further experiments were performed during 15 h just to analyse the first peak with 0.4 mg g^{-1} glucose. Fig. 3 shows the p–t curves due to the degradation of 0.4 mg glucose. Table 3 shows the corresponding thermodynamic parameters. It is interesting to note the shift of soil C t_{peak} with respect to those of soils A and B. Glucose as a carbon source induces all enzymatic systems involved in its

Table 2
Thermodynamic parameters as determined from the microbial growth p–t curves from Fig. 2 due to the degradation of (m_{cons}) mg g^{-1} glucose when 4.0 mg g^{-1} of the substrate was added to soil A with: (a) 4.7%, (a′) 12.9% and (A) 26.6% MC[a]

	A	a′	A
$-q_{obs}$ (J g^{-1})	19.68 ± 0.58	21.57 ± 0.97	34.37 ± 2.95
t_p (h)	8.54 ± 0.50	8.49 ± 0.13	41.08 ± 1.47
p_t (µW g^{-1})	163.50 ± 7.90	252.10 ± 14.40	492.50 ± 8.90
10^{-4} Δt (s)	13.81 ± 0.32	4.13 ± 0.15	3.73 ± 0.25
$-\Delta H'_{met}$ (kJ mol^{-1})	886.39 ± 26.12	971.51 ± 43.69	1548.02 ± 132.87
m_{cons} (mg g^{-1})	1.24 ± 0.04	1.36 ± 0.06	2.16 ± 0.19

[a] The growth peak occurs during the time interval (Δt) with a peak time (t_p) of height (p_t) producing a heat effect (q_{obs}) that corresponds to a metabolic heat $\Delta H'_{met}$.

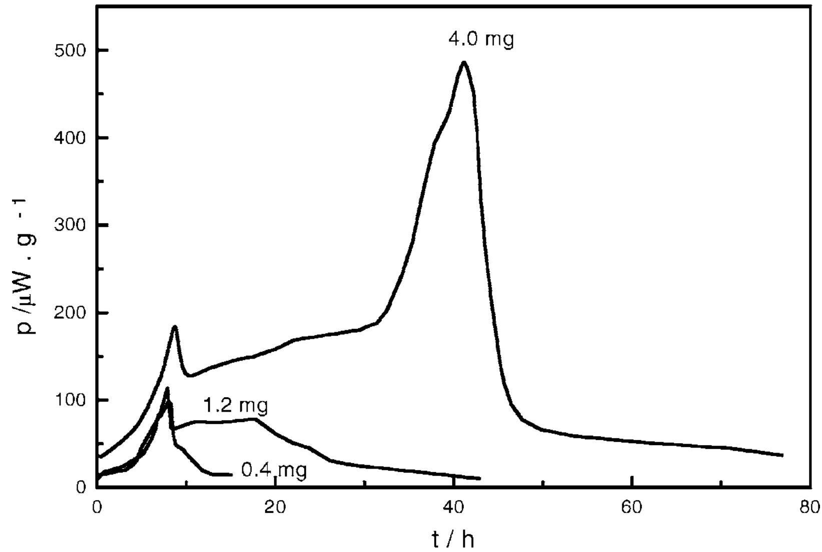


Fig. 2. Average specific thermal power–time curves for the degradation of 4.0, 1.2 and 0.4 mg glucose by the micro-organisms of soil A 2–3 years after deforestation at FCH.

consumption. When the carbon source is limiting as in the case of soil C, the enzymatic activity is delayed and the lag phase becomes longer and therefore the peak is shifted. For soils A and B, glucose concentration is not limiting due to the existence of other sources (i.e. OM) which is not the case for a poor soil as C. It should be noted that these soil samples come from the same 'Estancia' where crop production has always been soybean (summer) and wheat (winter). The only difference being the age of deforestation.

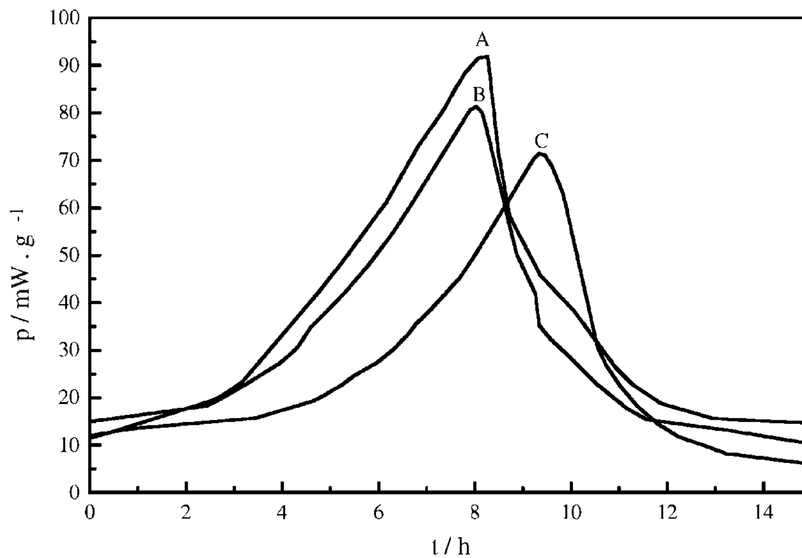


Fig. 3. Average specific thermal power–time curves for the degradation of 0.4 mg g^{-1} glucose by the microbial population of soil: (A) 2–3, (B) 5–6 and (C) 10–15 years after deforestation at FCH.

Table 3
Thermodynamic parameters as determined from the microbial growth p–t curves from Fig. 3 due to the degradation of (m_{cons}) mg g^{-1} glucose when 0.4 mg g^{-1} of the substrate was added to soil: (A) 2–3, (B) 5–6 and (C) 10–15 years after deforestation at FCH[a]

	A	B	C
$-q_{obs}$ (J g^{-1})	2.05 ± 0.16	1.69 ± 0.15	1.31 ± 0.13
t_p (h)	7.99 ± 0.11	7.99 ± 0.06	9.33 ± 0.01
10^{-4} Δt (s)	1.56 ± 0.05	1.58 ± 0.03	1.20 ± 0.07
p_t (μW g^{-1})	97.01 ± 0.80	80.69 ± 5.01	71.35 ± 5.59
$-\Delta H'_{met}$ (kJ mol^{-1})	923.32 ± 72.06	761.18 ± 67.56	590.02 ± 58.55
m_{cons} (mg g^{-1})	0.13 ± 0.01	0.11 ± 0.01	0.08 ± 0.01

[a] The growth peak occurs during the time interval (Δt) with a peak time (t_p) of height (p_t) producing a heat effect (q_{obs}) that corresponds to a metabolic heat ($\Delta H'_{met}$).

The q_{obs} values are directly correlated with the OM values through the following equation:

$$q_{obs} = -0.69\text{–}32.7 \times \frac{\text{OM}}{100}, \quad r^2 = 0.99 \qquad (6)$$

On the other hand, the product between p_t and the peak amplitude (Δt) is directly correlated with CFU g^{-1} values as determined in TSA for soils A and B and in agar-agar for soil C through the following equation:

$$p_t \times \Delta t = 1.93 - 1.36 \times 10^{-7} \times \text{CFU g}^{-1}, \quad r^2 = 0.98 \qquad (7)$$

These latter correlations are very interesting in cases of soil remediation. One could monitor the state of the soil just by running microcalorimetric experiments under the same conditions of the calibration curve determination.

From the results shown above, it is clear that soil C after 15 years since deforestation with continuous cultivation is almost completely worn out. Thus, we decided to perform some experiments on soil C samples with a composite made out of worms (Nutrilomb-20), which is sold as a foliage fertiliser and fungicide but farmers are using it as soil fertiliser with very good

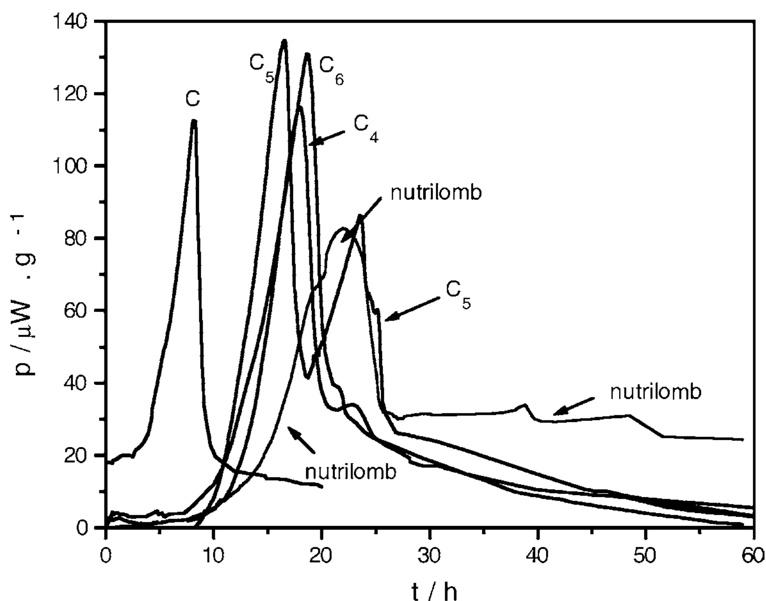


Fig. 4. Average specific thermal power–time curves for the degradation of 0.5 mg g^{-1} glucose by the microbial population of soil: (C) 10–15 years after deforestation, nutrilomb and autoclaved soil (C): (C_4) control, (C_5) inoculated with nutrilomb, (C_6) inoculated with sterile nutrilomb.

Table 4
Thermodynamic parameters as determined from the microbial growth *p–t* curves from Fig. 4 due to the degradation of (m_{cons}) mg g^{-1} glucose when 0.5 mg g^{-1} of the substrate was added to nutrilomb and to soil (C) 10–15 years after deforestation, inoculated soil (C): with nutrilomb (C_1), with sterile nutrilomb (C_2) and control (C_3), nutrilomb and sterile soil C: (C_4) control, (C_5) inoculated with nutrilomb, (C_6) inoculated with sterile nutrilomb[a]

	Nutrilomb	C, C_1–C_3	C_4	C_5	C_6
$-q_{obs}$ (J g^{-1})	6.13 ± 0.01[b]	2.15 ± 0.05	3.69 ± 0.15	5.35 ± 0.49	4.62 ± 0.14
t_p (h)	21.93 ± 0.57	8.30 ± 0.20	17.92 ± 0.29	16.58 ± 0.19	18.58 ± 0.06
				23.52 ± 0.67[c]	
10^{-4} Δt (s)	2.41 ± 0.39	1.13 ± 0.14	1.48 ± 0.09	1.37 ± 0.08	1.74 ± 0.04
p_t (μW g^{-1})	82.70 ± 5.90[b]	107.10 ± 5.80	116.20 ± 6.10	134.60 ± 16.60	130.90 ± 0.60
				86.20 ± 18.00[c]	
$-\Delta H'_{met}$ (kJ mol^{-1})	2208.76 ± 3.60	774.69 ± 18.02	1329.58 ± 54.05	1927.71 ± 176.56	1664.68 ± 50.44
m_{cons} (mg g^{-1})	0.39 ± 0.01[b]	0.13 ± 0.00	0.23 ± 0.03	0.34 ± 0.03	0.29 ± 0.01

[a] The growth peak occurs during the time interval (Δt) with a peak time (t_p) of height (p_t) producing a heat effect (q_{obs}) that corresponds to a metabolic heat ($\Delta H'_{met}$).
[b] Values per ml of nutrilomb.
[c] Corresponds to the second growth peak.

results. Samples (15% MC) were inoculated with this material as explained in Section 2 with a concentration equivalent to that used in the field (5% solution and 0.1 l m^{-2}). Sterilisation of samples did not eliminate all micro-organisms contained in them. Values of CFU g^{-1} of $(1.62 \pm 0.30) \times 10^7$ in the non-treated (autoclaved) samples were reduced to $(5.4 \pm 0.95) \times 10^6$ after treatment. CFU g^{-1} value in agar-agar for nutrilomb was $(2.01 \pm 0.02) \times 10^5$. Determined OM values were the same for treated and non-treated samples, $1.79 \pm 0.07\%$. Analysis of OM for nutrilomb showed a negative result with respect to easily oxidizable material. Calorimetric *p–t* curves for samples C, C_1–C_3 (C: original sample, C_1: inoculated with nutrilomb, C_2: inoculated with sterile nutrilomb and C_3: control) were identical whereas differences were observed for samples C_4–C_6 (C_4: autoclaved control, C_5: autoclaved, inoculated with nutrilomb and C_6: autoclaved, inoculated with sterile nutrilomb) as shown in Fig. 4. It is clear from curve C_4 of Fig. 4 that the microbial population contained in the soil samples subjected to heat and pressure stress were not completely killed. On addition of glucose, micro-organisms need more time to adapt (lag phase) than those involved in the curves of soil samples C and C_1–C_3. Note how the lag phase is coincident with the growth peak of the curve for soil sample C. Soil inoculated with nutrilomb (C_5) shows two growth peaks (16.6 and 23.5 h). The latter peak corresponds to that of the nutrilomb (22.3 h) whereas the first peak is almost coincident with that of the control curve, C_4 (18 h). In the case of C_6, the growth peak is also coincident with that of the control. Calorimetric control experiments run by amending the soil with different amounts of nutrilomb were not different from those of the baseline (see Table 4).

4. Conclusions

The *p–t* curves shown in this work clearly reflect the growth characteristic of the micro-organisms contained in the three soils studied and in the inoculated soil C samples. Apparently, as soil becomes worn out after years of deforestation and continuous crop production, the *autochthonous* microflora decreases. This is reflected in the *p–t* curves obtained when amending the soil with 4.0 mg of glucose (not shown for soils B and C). The peak at 41 h decreases from soils A–C. Therefore, the micro-organisms reflected in the calorimetric *p–t* curves at limiting carbon concentration are those less adapted to periods of low nutrient availability probably, of the *zymogenous* or *copiotroph* type. On sterilisation of soil (under the conditions used for this work), it is possible that these micro-organisms that are also poorly adapted in dealing with stress are eliminated remaining the populations that possess slower growth rates. To these latter populations belong several actinomycete species that produce long-lived

spores and other survival structures [1]. It seems that nutrilomb contributes to this latter type of microflora (see curve C_5 in Fig. 4) and therefore, farmers find that the product acts as a good soil fertiliser.

Acknowledgements

This work has been supported by a grant of the National University of Tucumán Research Council (CIUNT).

References

[1] D.M. Sylvia, J.J. Fuhrmann, P.G. Hartel, D.A. Zuberer (Eds.), Principles and Applications of Soil Microbiology, Prentice-Hall, Englewood Cliffs, NJ, 1998.

[2] U. Mortensen, B. Norén, I. Wadsö, Microcalorimetry in the study of the activity of micro-organisms, Bull. Ecol. Res. Commun. (Stockholm) 17 (1973) 189–197.

[3] S.A.M. Critter, J. de A. Simoni, C. Airoldi, Microcalorimetric studies of glucose degradation in some Brazilian soils, Thermochim. Acta 232 (1994) 145–154.

[4] N. Barros, I. Gomez-Orellana, S. Feijoó, R. Balsa, The effect of soil moisture on soil microbial activity studied by microcalorimetry, Thermochim. Acta 249 (1995) 161–168.

[5] K. Ljungholm, Ph.D. Thesis, University of Lund, Sweden, 1979.

[6] N. Barros, S. Feijoó, R. Balsa, Comparative study of the microbial activity in different soils by the microcalorimetric method, Thermochim. Acta 296 (1997) 53–58.

[7] A.G.S. Prado, C. Airoldi, The influence of moisture on microbial activity of soils, Thermochim. Acta 332 (1999) 71–74.

[8] R.B. Kemp (Ed.), Handbook of Thermal Analysis and Calorimetry, Vol. 4, Elsevier, Amsterdam, 1999, 391 pp.

[9] M.L. Jackson, Análisis Químico de Suelos, Ediciones Omega, S.A., 1970.

[10] R.K. Dart, Microbiology for the Analytical Chemist, The Royal Society of Chemistry, 1996.

[11] E.E. Sigstad, F.E. Prado, A microcalorimetric study of *Chenopodium quinoa* Willd. seed germination, Thermochim. Acta 326 (1999) 159–164.