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The influence of some physicochemical parameters on the microbial growth in soils☆

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Abstract

Microcalorimetric techniques have been used to study the influence of different physicochemical parameters on microbial growth in different soils in Galicia (NW Spain). The study was carried out using a 4 channels Thermal Activity Monitor (Thermometric, Sweden). Two types of soil, humic cambisol and umbric regosol, with different uses, vineyard, orchard, maize field, and scrubland, were investigated. Microbial activity in all the soils studied was stimulated by the addition of 1.25 mg of glucose g^{-1} soil. The power–time curves recorded from every experiment were analysed and from these analyses characteristic parameters such as peak time, peak height and microbial growth rate constants were determined. The influence of different environmental parameters, temperature (ambience and soil), moisture content (sample and residual), pH in water, and C/N ratio, were considered.

Keywords: Soil microcalorimetry; Microbial growth; Environmental parameters

1. Introduction

The potential productivity of a soil is closely related to its microbial load. At the same time, this microbial load can be used as an index to indicate soil degradation as a consequence of different factors going from an intensive and inappropriate agricultural exploitation to diverse contamination phenomena and/or forest fires. Because of this, the techniques used to study microbial activity can be very helpful to analyse the evolution of microbial population in soils under different situations. Microcalorimetry has shown as an important tool in the study of microbial activity in soils and its use is increasing nowadays [1–5].

Microcalorimetry has been successfully employed to study metabolism and microbial growth in soils as it permits the continuous monitoring of the activity of a living process in situ for a prolonged period without disturbing the system [5–9]. It is an useful tool for evaluating the metabolism of microbial biomass in soils because the heat produced in the various processes depends solely on the initial and final energy states of the system, and is independent of the types of micro-organisms and their form of evolution. Nevertheless it has rarely been used to study microbial

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growth in soil because of the practical difficulties of obtaining results for such a heterogeneous system [4,5,10,11].

Microcalorimetric measurements have generally been made using a close ampoule method which causes a decrease in the available O_2 with a corresponding CO_2 enrichment. Consequently, the environmental conditions change inside the closed ampoule [2].

In this paper, we investigated the influence that the concentration of a carbon source (glucose) has on the microbial activity in soils [2–4]. With this aim, six soils were collected in four different zones situated in Galicia (NW Spain). These soils were different both in use and origin.

2. Experimental

The main objective in field sampling was to collect a representative soil material for two types of soils, humic cambisol and umbric regosol, with different uses: vineyard, orchard, maize field, and scrubland (soils recovered using mining residues and covered by young bushes). It was intended to study how the use of soil influence on the global microbial population. Soil samples were collected from different rectangular plots of land (40 m^2 size) situated in the northern part of Galicia: Leiro (1a y 1b) and As Pontes (2a y 2b) in A Coruña, and two in the southern part: Cambados (3) and Porto do Cordeiro (4) in Pontevedra. The usefulness of the experimental measurements depends greatly on the samples being representative [2–4].

After sampling, soils were characterised by measuring different parameters such as: water-holding capacity, C, N, pH, and water content. For each of the six soils, the sampling was made from 10 randomly chosen points from each site. The samples were collected from a depth of 5–15 cm, after removal of surface material. The samples taken from each site were then carefully mixed in order to achieve a bulk sample representative of the soil to be studied. This bulk sample was placed in polyethylene bags, to reduce moisture losses, and returned to the laboratory. The soil was then sieved (mesh size $2 \times 2\,mm^2$) and the water content determined gravimetrically after oven drying at 105–110 °C to constant weight. Soil organic matter was measured as total C. Soil pH was determined using a Crison pH-meter. The measurements were performed introducing the electrode in supernatant solution prepared using 10 g of soil and 25 ml of water. Total nitrogen was determined by Kjeldahl, and water-holding capacity was determined by using a glass tube fitted with a fritted glass disc in the bottom being immersed in water [5]. The remaining bulk sample was stored in polyethylene bags at 4 °C for up to 3 months [2] before being used for the calorimetric experiments to ensure reproducibility of measurements.

Table 1
Main environmental parameters corresponding to the different sampling zones

Month	Northern zone (Leiro and As Pontes)				Southern zone (Cambados and Porto do Cordeiro)			
	Temperature (°C)	Rainfall (mm)	Evapotranspiration (mm)	Hydric availability (mm)	Temperature (°C)	Rainfall (mm)	Evapotranspiration (mm)	Hydric availability (mm)
January	6.6	222	16	322	10.0	227	20	327
February	6.8	194	23	294	10.8	177	30	277
March	9.0	161	47	261	12.6	144	60	244
April	9.9	120	69	220	14.3	122	81	222
May	12.5	112	97	212	16.0	131	108	231
June	15.1	75	108	175	19.1	61	133	161
July	17.2	35	122	102	21.0	31	152	59
August	17.3	63	101	63	20.7	47	115	47
September	16.2	102	58	102	18.7	115	56	115
October	13.3	146	39	191	16.2	149	47	208
November	9.4	198	17	298	12.8	201	16	301
December	7.1	255	15	355	10.1	195	15	295

Calorimetric experiments were performed using a microcalorimeter 2277 thermal activity monitor (Thermometric AB, Sweden), which is a commercial version of that developed by Suurkuusk and Wadsö [12]. Measurements were carried out in hermetically sealed 5 ml stainless steel ampoules. Soil samples of 1 g size at water-holding capacity were treated with 1.25 mg of glucose g^{-1} soil. Experiments at each concentration were repeated five times. The reference ampoule was filled with 1 ml of distilled water [2,13,14]. We have found that the results obtained by doing this agree reasonably well with those obtained using a soil as reference [2–4,14–17].

3. Results and discussion

Main climatic characteristics of the four zones studied are shown in Table 1. Environmental conditions are key on the formation and evolution of soils. Because of this, the data here shown are very important in order to analyse and understand the influence of environment on physicochemical characteristics of soils and on the microbial population.

All the experiments were carried out at 25 °C. Microbial growth rate constant, μ, was quantified from the power–time curves recorded by the calorimeter. This value is only an apparent one and does not give information about the biochemical activity of the

Table 2
Physicochemical properties of the different soils studied

Zone and use	Temperature (°C)		Moisture (%)		Field capacity (%)	C/N	pH
	Soil	Ext.	Sample	Residual			
(1) Leiro							
Humic cambisol							
(1a) Vineyard	14.8	21.9	6.26	1.67	18.84	10.63	4.56
(1b) Orchard	15.7	23.8	3.91	1.78	17.34	10.97	6.39
(2) As pontes							
Humic cambisol							
(2a) Vineyard	17.8	22.0	10.91	2.03	19.67	10.25	5.54
(2b) Maize field	18.1	21.5	15.76	2.51	18.62	11.00	5.72
(3) Cambados							
Umbric regosol	14.5	20.0	13.36	4.98	32.14	17.47	4.53
(4) Porto do Cordeiro							
Umbric regosol	15.5	21.2	21.98	4.03	27.39	13.49	3.76

Table 3
Main calorimetric parameters obtained from microcalorimetric experiments

	Soils					
	1a	1b	2a	2b	3	4
Q_t[a] ($J\,g^{-1}$)	3.35 ± 1.10	2.36 ± 0.46	3.40 ± 0.27	1.81 ± 0.94	2.47 ± 0.70	1.53 ± 0.75
P_t[b] (h)	15.05 ± 0.36	14.32 ± 1.11	11.05 ± 5.63	8.30 ± 0.43	19.23 ± 0.66	26.26 ± 0.69
Qp_{height}[c] ($J\,g^{-1}$)	0.55 ± 0.33	0.75 ± 0.20	1.08 ± 0.50	1.30 ± 0.47	1.55 ± 0.46	0.98 ± 0.45
μ[d] (h^{-1})	0.438 ± 0.134	0.337 ± 0.041	0.451 ± 0.027	0.641 ± 0.082	0.285 ± 0.089	0.126 ± 0.029
R[e]	0.985 ± 0.016	0.997 ± 0.003	0.997 ± 0.001	0.998 ± 0.001	0.988 ± 0.008	0.991 ± 0.002

[a] Total heat evolved up to the peak of the power–time curve.
[b] Time to reach the peak.
[c] Heat in the peak.
[d] Microbial growth rate constant.
[e] Correlation index.

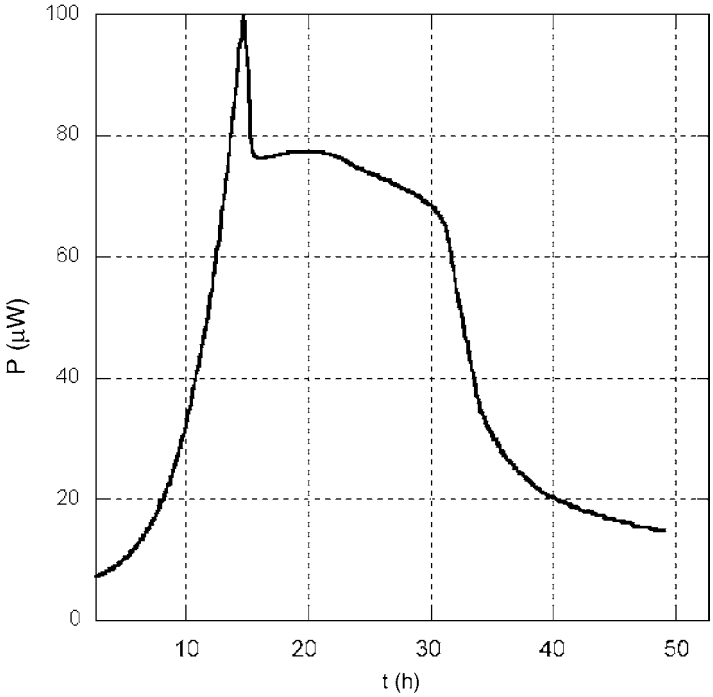

Fig. 1. *P* (μW)–*t* (h) plot: soil 1a (vineyard).

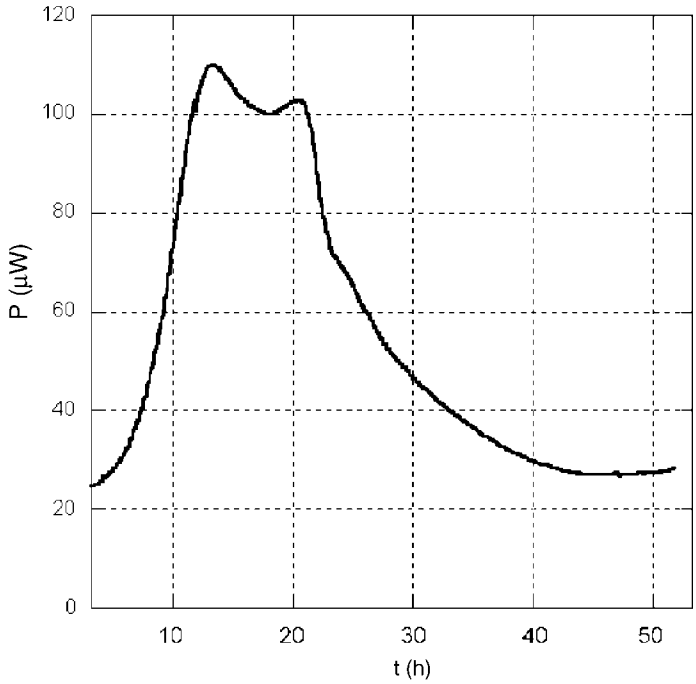

Fig. 2. *P* (μW)–*t* (h) plot: soil 2a (vineyard).

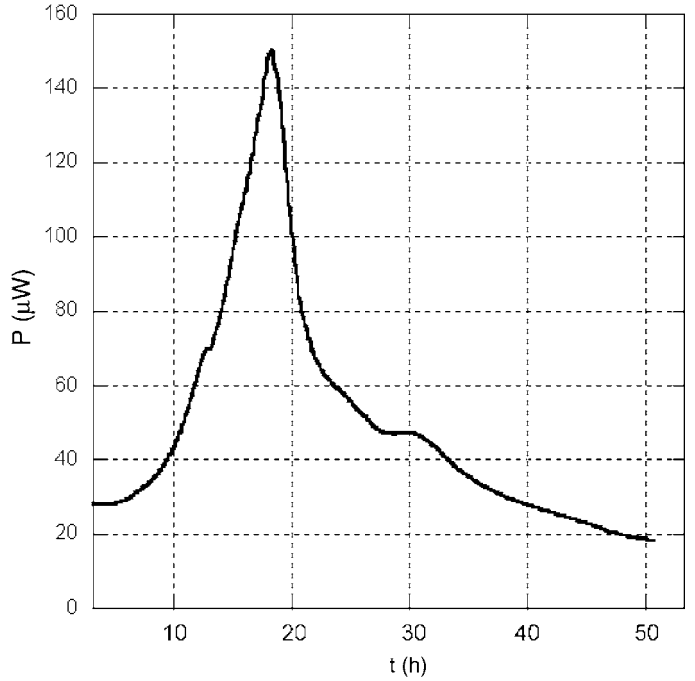

Fig. 3. *P* (μW)–*t* (h) plot: soil 1b (orchard).

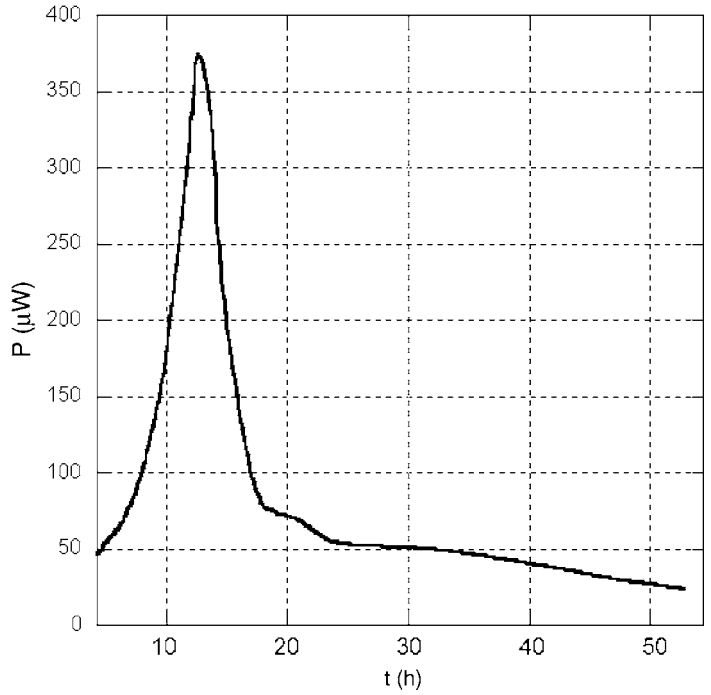

Fig. 4. *P* (μW)–*t* (h) plot: soil 2b (maize field).

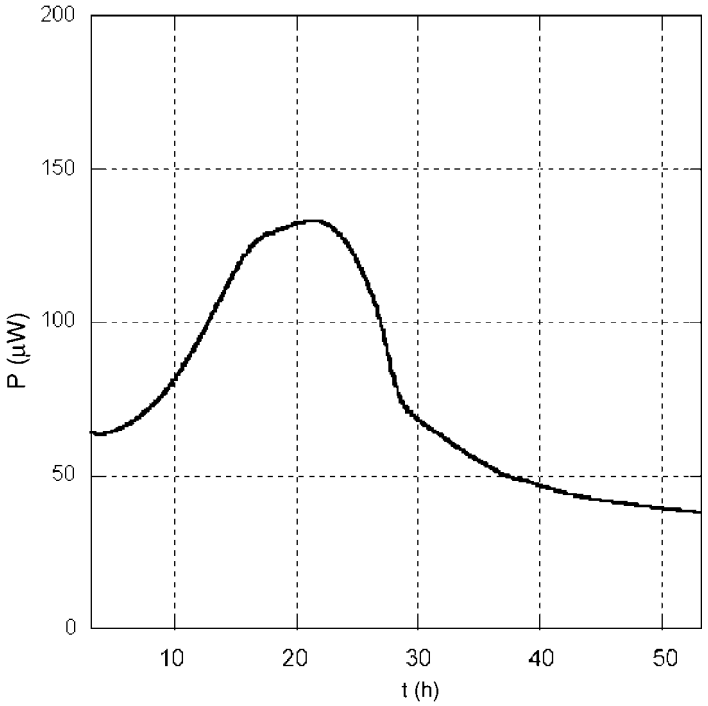

Fig. 5. *P* (μW)–*t* (h) plot: soil 3 (scrubland).

individual microbes. However, since heat evolution is also proportional to the amount of glucose degraded, it can reasonably be considered as the specific degradation rate of glucose under the given conditions in soil, and may be used as an index to express how fast the material is decomposed by microbial action [1].

Main physicochemical properties of the different type of soils studied are listed in Table 2. From the analysis of this table it follows:

1. All the soils studied show strong acid pH values. This could be a consequence of their high organic matter load (>4%) and also of the fact that they are excessive washed by abundant rains. Moreover, these soils originated from granite. It must be pointed out that the value pH = 6.90 corresponding to soil 1a is due to the traditional use of lime, very common in the treatment of agriculture soils in Galicia. This is corroborated by the fact that the main part of vegetal species growing in Galicia show the need for neutral pH. On the opposite, it must be underlined the value 3.76 corresponding to soil 4. This value is probably due to soil composition, on the one hand mining residues and, on the other hand, organic matter in the onset of decomposition thus originating a great amount of fulvic and humic acids as a previous stage to “stabilisation” and generation of humus.
2. The ratio soil/environment temperatures supplies a valuable information about soil structure. In this way, it can be seen that degraded (low structured and highly compacted) soils 3, 4 show temperatures very similar to environment temperature. The reason is that the lack of structure hinders an appropriate regulation effect of soils before temperature changes [18–20]. However, soils 1a and 1b better structured and also protected by agriculture tasks show temperatures well differentiated from environment. Soils 2a and b, show also very similar temperatures. However, in this case, the similarity of temperatures was based on the fact that sampling was made immediately after seasonal crop collection and subsequent plough up, thus becoming cleared soils.

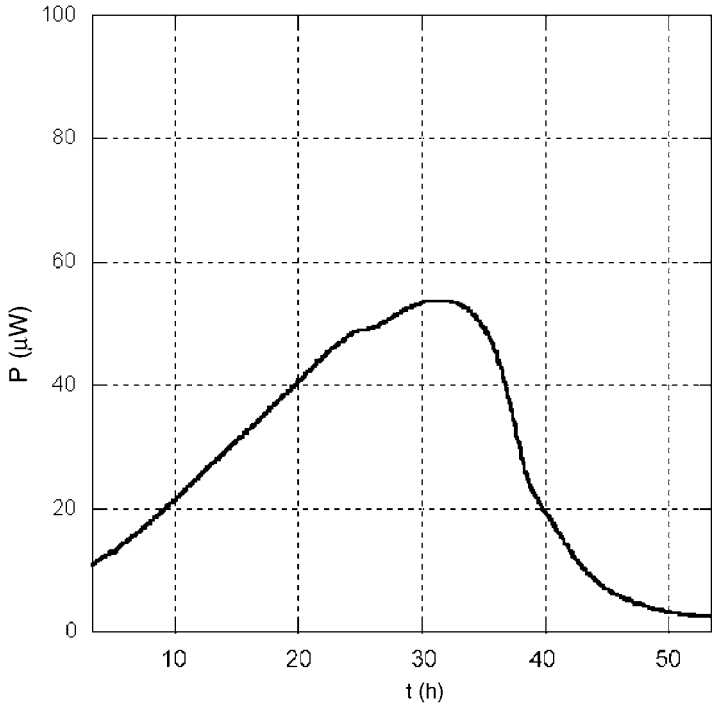

Fig. 6. *P* (μW)–*t* (h) plot: soil 4 (scrubland).

3. As expected, moisture contents both that corresponding to the sample and the residual moisture are lower for cultured soils as a consequence of an adequate drainage necessary to increase productivity, while scrubland soils show greater moisture contents. High moisture content conditions can originate anaerobiosis during long periods over the year and, as a consequence, the dead of aerobic micro-organisms. In this case, the growth rate constant would show lower values.
4. A similar discussion can be made for field capacity, as the well-structured soils show lower values than those corresponding to compacted soils.The ratio C/N, very important to determine the degree of mineralisation, is again greater for soils 3 and 4 as a consequence of their low degradation and, also of the composition of these soils originating from mining residues. The high value of these ratios constitute a hindering to vegetative species in these kind of soils, thus favouring the growing of minor species such as erica spp. and bushes.

The influence of each parameter on microbial growth was considered separately as these parameters depend both on the environmental conditions and the use of soil, that change seasonally over the year. The reason for considering separately each of the factors is to avoid overlapping that could obscure our study. By doing so, the response of the microbial community to the stimulus caused by changes in the different seasonal parameters can be analysed. Anyway, as it is well known, all these parameters are interrelated when considering environmental phenomena.

Table 3 shows results obtained from the calorimetric experiments performed. As expected, values of the growth rate constant are lower for scrubland soils 3 and 4 as a consequence of their low structure, high compaction, high moisture content, very changeable thermal regime and a high dependence on environment. These soils show also a very poor variety of vegetative species, thus conditioning the relationship between micro-organisms and soil, because, as it is known, the greater the vegetal species variety, the greater the amount and variety of micro-organisms as

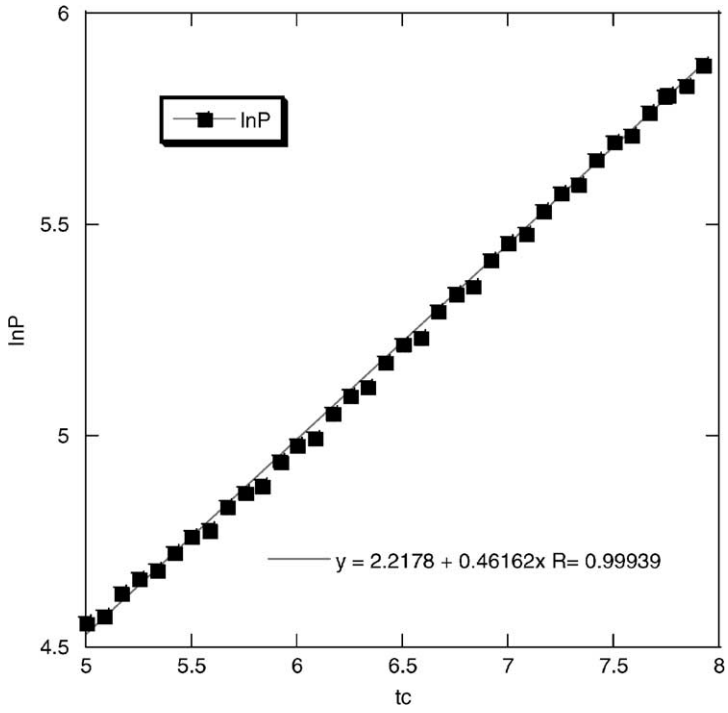

Fig. 7. Plot of $\log P_t$ versus time obtained from the semilogarithmic conversion of power–time curves recorded from soil sample number 3.

it can be seen analysing values of thermal power obtained from the microcalorimetric experiment. On the opposite, soils 1a, 2a and 2b show high μ, being the last one the highest value of μ amongst all the soils studied, as a consequence of the addition of fertilisers. The low value of μ corresponding to soil 1b could originate from the fact that this soil was left fallow after being overexploited for years.

Figs. 1–6 show power (μW)–time (h) plots. As it can be seen, couples 1a and 2a, 1b and 2b, and 3 and 4, respectively, show similar shape. These similarities could be understood in terms of the use given to the different kinds of soils. All of them show, more or less, the different phases of latency, exponential growing, steady phase and decay.

Fig. 7 shows a power–time plot obtained from the semilogarithmic conversion of the curves recorded from a soil sampling containing 1.25 mg of glucose g^{-1} soil. The resulting straight line is a proof of the exponential behaviour of the thermal power during the microbial growth induced by a carbon source. The microbial growth rate constant was calculated from the slopes of the different straight lines. All the other experiments show a behaviour similar to soil 3.

4. Conclusions

Microcalorimetric technique shows as a suitable tool to study microbial growth in soils, the study can be made taking into account the physicochemical and biological properties of soils.

Cultivated soils show a greater microbial activity than soils not cultivated. Cultivated soils keep their properties nearly constant in values considered as optima for a good soil behaviour both from the physicochemical (pH, temperature, CO_2/O_2 ratio, C/N ratio, moisture content, etc.) and biological (nutrients, organic matter, etc.) points of view. Because of this, a rational exploitation of soils can allows a sustainable and stable microbial population thus indicating the maintenance of soil productivity. Degraded soils present low μ values.

Analyses the power–time curves lead to an understanding of microbial activity in similar soils, as it can be seen in Figs. 1–6.

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