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STUDY OF THE INFLUENCE OF DIFFERENT FOREST SPECIES ON THE MICROBIAL ACTIVITY IN SOILS

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Microcalorimetry was used to study the seasonal evolution over one year of the microbial activity in a humic-eutrophic Cambisol soil as a function of its forest cover. The study was carried out on three soils with identical origin but covered with different forest species: pine, eucalyptus, and a typical Atlantic-humid riverside forest.

Some other physical, chemical and biological properties and environmental parameters, mainly humidity and environmental temperature, were considered to analyze their influence on soil microbial activity.

The study was performed using a microcalorimeter Thermal Analysis Monitor 2277 in which the experiments were carried out with 1 g soil samples treated with 1.25 mg glucose g^{-1} soil. From the measured results it follows that pine forest soil is the least productive of the three, as it generates an average heat of 2.7 *vs.* 5.9 J g^{-1} generated by the eucalyptus forest soil and 3.1 J g^{-1} generated by the riverside forest soil. These results are dependent on the remaining physical, chemical and biological features analysed and because of this, pine forest soil, with a pH value 3.3 in spring, shows a small capacity to maintain a stable microbial population which is the lowest of the three ($0.079 \cdot 10^8$ to $0.46 \cdot 10^8$ microorganisms g^{-1} soil) while riverside soil microbial population is in the range from $7.9 \cdot 10^8$ to $17 \cdot 10^8$ microorganisms g^{-1} soil.

Keywords: *forest cover, microbial activity, microcalorimetry, rational and sustainable exploitation*

Introduction

In the last 10 years, the European Union (EU) has elaborated many environmental plans focused on the protection and maintenance of the environment under the premises of a rational and sustainable development as a basis for the preservation of the current-technological society. The protection of soil and its resources is one of the priority tasks of the present world welfare society as it is the point of convergence of energy, agricultural, environmental, economical and social policies. Soil is not only a physical mean in which our communities build houses, but also the direct or indirect origin of most part of the products used in our daily diet, and also a highly complex system for storage and purification of water [1].

In this article, a study of the influence of different forest formations on soil quality is made. Soil quality is understood as the convergence of physical, chemical and biological features that have influence on its productivity as a consequence of the use at which is subjected under the action of climatic and environmental conditions.

The study is focused on the effect of three forest formations on a soil using microcalorimetry as the experimental technique and microbial growth as a bioindicator of soil quality [2–8]. With this purpose, a zone where the three forest formations grow, situated in Viveiro (Galicia, NW Spain), was chosen. The soil in the whole zone has a common origin, humic-eutrophic Cambisol, and it is subjected to similar climatic conditions.

- Soil 1. An area covered by eucalyptus, mainly *E. globulus* Labill. The soil was not subjected to silviculture tasks during the last 100 years. Eucalyptus is a fast growing species and is extremely aggressive against soils mainly at first stages of growing as it extracts huge amounts of nutrients and water.
- Soil 2. An area covered by pines, with a stable forest biomass for the last 60 years. This forest species, *P. pinaster* Aiton, generates forest residues that on degradation produce great amounts of different acidic matters that constrict the growth of any other forest species.
- Soil 3. An area having a typical Atlantic-humid climate forest, where the main forest species consist of eucalyptus (most abundant species for the last 30 years), oaks, birchs and other riverside forest species, being the forest surface covered by vegetal litter combined with low habit herbaceous species.

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The experimental procedure here proposed is based on the following assumptions [9]:

- Soil productivity directly depends on main features that are common to every kind of soil: a) physical fertility; that is, the capacity to supply plants the necessary nutrients in the adequate form, proportion and time, b) own fertility, or the supply of the water, oxygen and heat necessary for vegetal growth, c) living phase in soil, who is the final responsible for soil productive potential.
- Microorganisms are real bioindicators of soil quality. Soil stress originated by a continuous exploitation reduces both the quantity and the diversity of microorganisms, thus diminishing the soil future richness.
- Soil living phase evolution, and thus the study of microbial activity in soils, can be quantified by using microcalorimetry.

On the assumptions that soil quality depends on physical, chemical and biological features and also on the environmental conditions, this study can be complemented through a deep analysis of soil physical (texture, structure, porosity and plasticity), chemical (pH, elementary composition, and C to N ratio) and biological properties (MPN or organic matter content) together with a study of the environmental properties (climate, vegetation and geological substrate) that have a strong influence on the aforementioned properties and, thus on the microbial activity in soil [10–12]. All the results calculated from different tests must be analysed together in order to obtain a real knowledge of the global soil behaviour.

Experimental

Materials and methods

For a better understanding, this study was divided into three very well differentiated parts: sampling phase, microcalorimetric study, and analysis of physical, chemical, biological and climatic environmental properties.

Sampling phase

To obtain a thorough knowledge of the evolution over the year of microbial activity in soils subjected to a highly changeable weather, as it happens in Galicia (NW Spain), samples were collected in four different seasonal periods.

The study begins by the choice of a zone having common geological substratum, humic-eutrophic Cambisol, subjected to similar climatic conditions. This choice pretends to establish a stable starting point that allows to consider the actions of forest formations as the only responsible of changes in soil behaviour. Three different land plots, 200 m far from each other, covered, as it was previously mentioned, by eucalyptus [8], pine trees and a riverside forest, were chosen. Each of the zones cover a minimum of 5000 m^2. Within each of the land plots, a sampling zone of 100 m^2 was taken, that were divided according to statistical criteria in 1 m^2 sites, being the samples collected from these sites. After the removal of the very top layer of soil, samples were collected to a depth of about 15 cm. All samples from one site were mixed and sieved. The sample was then reduced through a coning and quartering procedure. By doing so, the sample was highly homogenized, thus allowing to obtain reproducible and representative results [13] and showing the diversity and the density of the microorganisms existing in the environment where the sample was collected. The samples were introduced into polyethylene bags, to avoid contamination and loss of moisture, and then sent to the laboratory in less than 10 h. Using the experimental procedure here described, two different kinds of samples were taken, one for microcalorimetric measurements (400 g), named sample 1, and the other (10 kg) for determination of physical, chemical and biological features, named sample 2.

During the sampling stage, some environmental features such as: soil temperature, environment temperature and moisture content were measured, and also the whole zone was described: topography, vegetation, etc. and the soil apparent density was determined.

Calorimetric phase

Once in the laboratory, the moisture content was determined as the mass loss after drying of the sample in a Selecta 200210 natural desiccating oven, at 105°C, to constant mass [14]. The samples were also left to dry at a room stable temperature of 20°C, to determine residual moisture following the procedure previously described. Samples were sieved using a R72 (mesh size 2×2 mm^2) and the one to be used for calorimetric experiments, sample 1, of about 400 g, was placed in hermetically closed polyethylene bags and left in the laboratory, at 4°C, for up to 3 months [15] to ensure reproducibility of calorimetric measurements. The remaining sample, sample 2, of about 10 kg was used for determination of physical, chemical and biological features. After that time, calorimetric experiments were performed using a microcalorimeter 2277 Thermal Activity Monitor (TAM) Thermometric AB [16]. Measurements were carried out in hermetically sealed 5 mL stainless steel ampoules. This closed ampoule method causes a decrease in the available O_2 with a corresponding CO_2 enrichment [17]. Consequently, the environmental conditions inside the closed ampoule change. Soil samples of 1 g size at water-holding capacity were treated with 1.25 mg of glucose g^{-1} soil. This glucose concentration value was determined after

trying different concentrations [6]. Each experiment was repeated four times. The reference ampoule was filled with 1 mL of distilled water. It was found that the results obtained by doing this agree reasonably with those obtained using a soil as reference. Calorimetric results showing soil behaviour were reported in the form of power–time curves [8, 11, 12].

Other properties

This section includes all the normalized tests [8] used for determination of soil physical, chemical and biological properties as well as bioclimatic determination that are presented in the form of bioclimatic diagrams, Fig. 1 [18, 19]. These diagrams are very helpful for the understanding of the influence of climatological parameters on both the soil living phase and the soil productive capacity as a function of the vegetative activity of the vegetals growing in a particular zone. The different tests comprise:

- Physical properties: water-holding capacity determination, porosity, hydraulic conductivity, structure, texture, actual density, plasticity, adhesivity and optimum humidity content for compaction.
- Chemical properties: elemental composition, C to N ratio and pH.

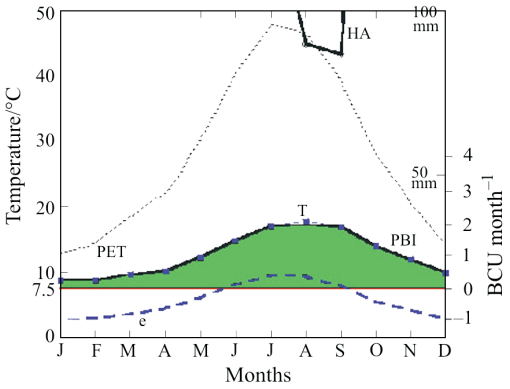

Fig. 1 Bioclimatic diagram [8, 18, 19] of the sampling zone

- Biological properties: MPN and organic matter content.

All the experiments were carried out at atmospheric pressure, 20°C and a relative humidity of 75%. All properties, except for pH, temperature and moisture content, were determined once a year.

Analysis of all soil properties as a whole gives a very important partial information about soil current physical state, and can be used as a basic reference for understanding of soil potentiality, or health state, when the study is complemented through microcalorimetric data.

Results and discussion

Table 1 records values of physical, chemical and biological properties of the three soils studied. From these values, the productive potential in soils can be estimated:

- The soil covered by pine forest shows a low pH [20, 21] over the whole year, mainly in spring. This very acid pH originates very unfavourable conditions for microorganisms developing and, except for Fe, the assimilation of the elements that plants need for growing (Cu, Zn, B, Mn, K, Mg, Mo, Ca, P and S) is very poor or none. Despite Galician soils are, in general, acid soils as a consequence of the interaction of many factors such as: mineralogic origin (granites, sandstone, and schist), high content in organic matter, and high hydraulic conductivity, soil 2 shows an extra cause for pH lowering and that is the formation of acidifying substances during the degradation of forest residues generated from pine trees. The remaining soils, eucalyptus and riverside forest cover, even they are strongly acid with a pH mean value of 5.0, allow a better development of microorganisms thus showing greater MPN values and because of this a greater microbial activity and also a greater productive potential.
- Both structure and texture of the three soils are very good with a very good thermal stability over the year with a mean temperature of 14°C. For this reason, temperature that is, together with moisture content and pH, one of the basic features for a correct microbial activity is not a limiting factor for any of the three soils.
- Soil moisture content should be analyzed together with water-holding capacity, hydraulic conductivity, and porosity in order to understand its importance. At first, moisture content is high in soils with low hydraulic conductivity and high water-holding capacity. It could seem strange that the soil occupied by eucalyptus forest with a porosity of 60.5% presents a conductivity of $8.9 \cdot 10^{-3}$ m s^{-1}, while pine and riverside forest soils with porosities of 77.4 and 67.5% present hydraulic conductivites of $4.1 \cdot 10^{-3}$ and $5.3 \cdot 10^{-3}$ m s^{-1} respectively, both lower than the eucalyptus one. This can be explained considering that this last soil has a surface horizon 1.5 m deep formed by forest residues in different phases of degradation. Moreover, this soil is highly hydrophobic and generates a very efficient conductivity.
- Values of real density are very similar, around 1600 kg m^{-3}, for the three soils as a consequence of their common origin. However, pine species generate a strong acid attack (chemical weathering) on soil, originating a fast degradation that makes soil more spongy. Because of this, pine forest soil has an apparent density of 300 kg m^{-3}, while eucalyp-

Table 1 Physical, chemical and biological properties corresponding to soils 1, 2 and 3

	Soil	Spring	Summer	Autumn	Winter
pH	1	4.3	4.8	5.3	4.2
	2	3.3	4.2	4.2	4.6
	3	5.1	5.7	5.6	5.0
Temperature/°C	1	13.1	17.2	15.2	11.8
	2	12.0	16.2	14.6	11.1
	3	12.3	16.5	14.3	10.5
Moisture/%	1	14.2	10.3	10.8	24.9
	2	28.9	23.1	32.9	37.4
	3	28.2	17.5	30.0	32.3
MPN (microorganism g^{-1} soil)	1	$4.00 \cdot 10^8$	$7.00 \cdot 10^8$	$4.70 \cdot 10^8$	$1.70 \cdot 10^8$
	2	$0.14 \cdot 10^8$	$0.46 \cdot 10^8$	$0.079 \cdot 10^8$	$0.13 \cdot 10^8$
	3	$17.00 \cdot 10^8$	$11.00 \cdot 10^8$	$9.50 \cdot 10^8$	$7.90 \cdot 10^8$

	Other physico-chemical properties		
	Soil 1	Soil 2	Soil 3
Texture	sandy loam	loam	sandy loam
Actual density/kg m^{-3}	1520	1490	1790
Apparent density/kg m^{-3}	600	337	582
Residual moisture/%	6.7	7.5	3.3
Total porosity/%	60.5	77.4	67.5
Air porosity/%	51.5	65.4	51.8
Water porosity/%	9.0	12.0	15.7
Plasticity index	little plastic	little plastic	little plastic
Adherence	grain	grain	grain
Proctor/%	31.1	30.0	21.5
Hydraulic conductivity constant, K/m s^{-1}	$8.9 \cdot 10^{-3}$	$4.1 \cdot 10^{-3}$	$5.3 \cdot 10^{-3}$
C/N	13	22	9
Organic matter	4.4	8.3	2.7
Field capacity/%	16.5	35.5	25.3
Structure	very good	very good	very good

Values of pH, T, M and MPN were calculated for each sampling, because they depend on climatic conditions that change over the year. The other properties were determined only once because they remain steady over the year providing the soil was not subjected to strong man activities

tus and riverside soils show an apparent density of 600 kg m^{-3} each.

- Residual moisture is closely related to the humidified organic matter content and to the soil water retention capacity. Pine forest soil shows a residual moisture of 7.5% with an organic matter content of 8.3%.
- Owing to texture and structure, Galician soils show low plasticity, adhesivity and compaction index. This is a direct consequence of the predominance of structuring and also of the predominance of cohesive over adhesive forces [22]. In this particular case soil needs a moisture content of 25% to reach the maximum compaction index, which is more than 10% higher than the value that most part of soils need for maximum compaction. This is a consequence of the predominance of the sand-lime over the clay fraction in Galician soils.
- As it is known, high values of the C to N ratio indicate the generation of soil humification processes [21, 23]. On the opposite, values lower than 5 denote an excessive soil mineralization that could destroy microbial soil population. Analysis of Table 1 shows that soil 3 with a C to N ratio of 9, that means an equilibrium between mineralization and humification, and thus a good fertility that needs from organic matter addition to mantain productive potential. C to N ratio values for the remaining 2 soils are greater than 12 thus showing a predominance of humification processes. It looks strange that soil 3 with the lowest C to N value is the one with the highest MPN. This fact can be explained on the basis of a fast consumption of the senescent organic matter by the very active microbial population, and also because the organic matter content of

this soil, main carbon source, is the lowest of the three soils. Values of the C to N ratio allow the identification of the type of humus as an acid forest mull, in the case of soils 1 and 3 (temperature climate neoformation humus with hardwood vegetation, mainly oaks) than trend to moder in soil 2 as a consequence of the land slope and the peculiar features of pine trees and their action on soil (similar to previous one but subjected to less washing).

- As it was previously mentioned, MPN is highly influenced by soil pH, that is a limiting factor both for growth and activity of microbial population. It can be seen that soil 2 shows the lowest MPN during the whole year that in autumn reaches a value of $0.079 \cdot 10^8$ microorganisms g^{-1} soil. On the opposite, soil 3 having an adequate pH over the year is the one showing the highest MPN, that in autumn becomes $9.5 \cdot 10^8$ microorganisms g^{-1} soil.
- Related to the previous point, it can be seen a parallelism between organic matter content and MPN. In fact, soil 3 has the highest MPN and the lowest organic matter content while soil 2 is the one with the greatest organic matter content and the lowest MPN. This seems very logical since having the lowest number of microorganisms, the comsumption of C is minimal and thus the organic matter accumulates. Even so, Galician soils, very rich in organic matter, can be considered as very productive.

Values of pH, temperature, moisture and most probable number of microorganisms (MPN) were calculated for each sampling, as they depend on climatic conditions that change over the year. The other properties were determined only once because they remain steady over the year providing the soil was not subjected to strong man activities.

Next step was the confirmation of the previous results through microcalorimetric experiments. Table 2 shows the main features analysed during the calorimetric study. These features are presented in Fig. 2:

- Q (J g^{-1}), total heat evolved during the whole process. As a thumb rule, the greater the microbial biomass the greater should be Q and thus the soil productive potential.
- P_t (h), is the time to reach the maximum of the peak. The smaller its value, the greater the soil response before external changes.
- Q_t (J g^{-1}), is the total heat evolved up to the maximum of the power–time curve. This feature combines the previous two.
- μ (h^{-1}), is the microbial growth rate constant. It is inversely related to P_t, as the greater is μ, the smaller is P_t (high μ values are related with high soil reactivity). When a soil has high μ values its capacity to react to different external perturbations (forestry or agricultural exploitation, pollution phenomena or its capacity to resist fires in forestry) is better.

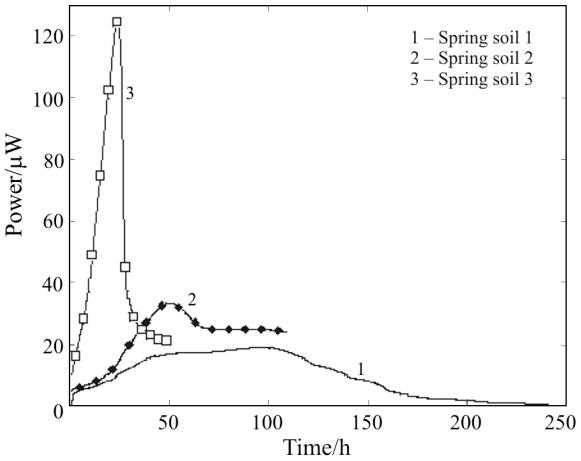

Fig. 2 Evolution of the three soils studied in spring. Every plot shows all the different microbial growth phases [12]: latency phase, exponential growth, steady phase, and death phase. Analysis of the plot shape allows prediction of soil behaviour

Values shown in Table 2 are the mean of 4 experiments carried out on each sample and season. From these values it follows:

- Soil 1 shows the greatest mean Q value over the year, while soil 3 shows the lowest one. However, when Q and MPN are analysed together some discrepancy appears. This apparent discrepancy can be justified considering that soil 1 has a P_t of about 210 h in summer, while soil 3 only 22 h. This could be due to soil 1 peculiarities and its difficulty to develop an adequate microbial activity from the forest residues originated by eucalyptus, that are strongly resistant to degradation and, up to certain point, relatively new for soils, as this forest species was introduced in Galicia only 150 years ago. Something common to the three soils is that all reach the highest Q value around spring–summer, that is the most adequate period for microbial activity as a consequence of the temperature values, while winter–autumn show low microbial activity because temperature is a limiting factor for this activity. These values are represented in Fig. 1, where it can be seen that the bioclimatic intensity potential is maximum in spring–summer, thus originating a propitious environment for vegetative development and soil microbial activity.
- P_t values must be analyzed together with μ values to understand soil reactivity, that is the demonstration of the adaptation to soil of the living microorganism in an environment without significant changes in the last 7500 years. As it can be observed, the lowest P_t coincides with the highest μ that correspond to soil 3 (typical Atlantic forest), while soil 1 (eucalyptus forest) shows an opposite

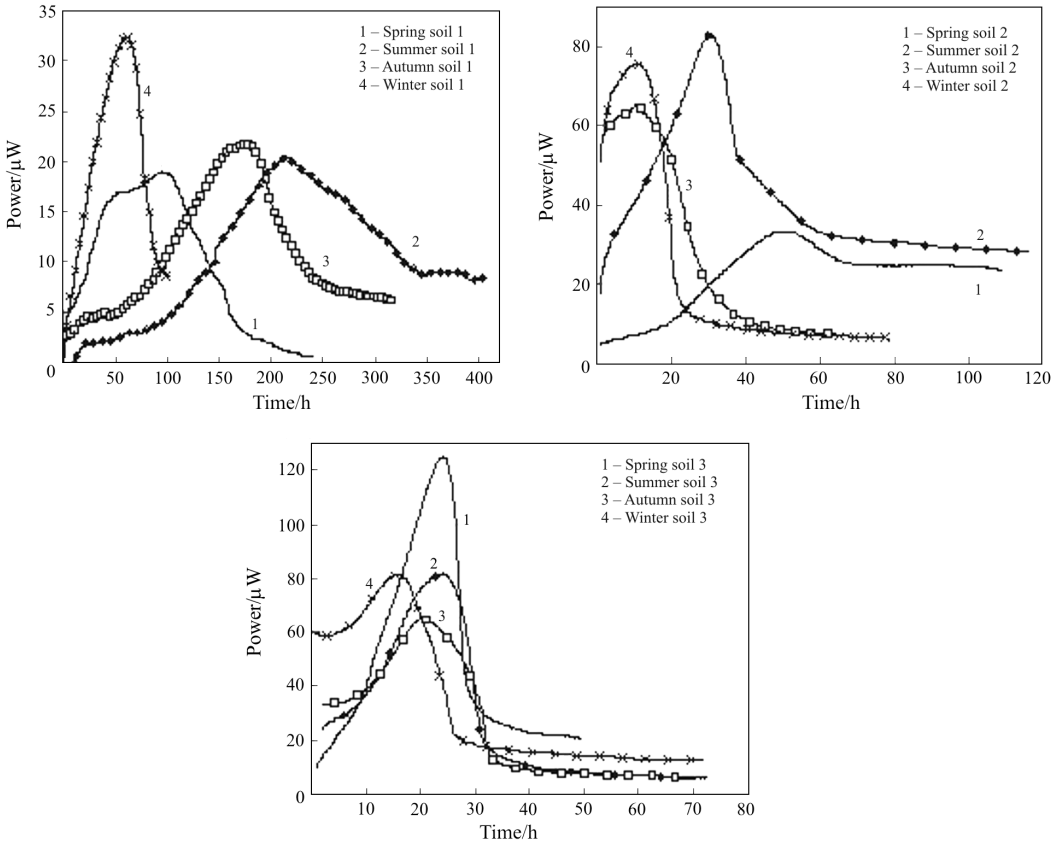

Fig. 3 *P–t* curves corresponding to microbial activity evolution in the three soils over the year

Table 2 Characteristic microcalorimetric parameters

	Soil	Spring	Summer	Autumn	Winter
Q/J g^{-1}	1	6.10±0.23 (3.77%)	6.85±0.02 (0.27%)	6.32±0.21 (3.26%)	4.48±0.06 (1.27%)
	2	2.35±0.05 (1.94%)	5.70±0.14 (2.49%)	1.32±0.05 (3.65%)	1.40±0.06 (3.98%)
	3	4.54±0.20 (4.31%)	3.53±0.13 (3.60%)	2.45±0.05 (2.20%)	1.73±0.05 (2.74%)
P_t/h	1	89.21±4.07 (4.56%)	209.61±4.49 (2.14%)	175.75±1.77 (1.01%)	67.29±0.30 (0.44%)
	2	55.19±1.15 (2.08%)	36.85±1.40 (3.80%)	10.63±0.34 (3.24%)	8.54±0.24 (2.82%)
	3	22.42±1.11 (4.94%)	22.33±0.59 (2.64%)	20.27±0.24 (1.18%)	17.75±0.82 (4.62%)
Q_t/J g^{-1}	1	3.45±0.08 (2.19%)	2.80±0.11 (3.76%)	3.29±0.12 (3.53%)	3.08±0.11 (3.73%)
	2	1.35±0.05 (3.71%)	2.59±0.11 (4.36%)	0.36±0.01 (3.17%)	0.33±0.01 (2.95%)
	3	3.24±0.07 (2.06%)	2.14±0.06 (2.63%)	1.05±0.03 (3.20%)	0.91±0.01 (1.31%)
μ/h^{-1}	1	0.054±0.0010 (1.82%)	0.0017±0.0010 (2.74%)	0.0012±0.00001 (1.09%)	0.0117±0.0003 (2.57%)
	2	0.0495±0.0017 (3.49%)	0.0292±0.0013 (4.38%)	0.0104±0.0004 (3.45%)	0.0052±0.0002 (4.54%)
	3	0.0750±0.0013 (1.77%)	0.0531±0.0020 (3.83%)	0.0423±0.0019 (4.59%)	0.0309±0.0010 (3.38%)

Q/J g^{-1} is the total heat evolved during the processes; peak time, P_t/h is the time to reach the maximum of the peak; Q_t/J g^{-1} is the total heat evolved up to the maximum of the Power-time curve; μ/h^{-1} is the microbial growth rate constant. The final result is 6.14±0.23 (3.75%), where 6.14 is the mean value calculated from 4 microcalorimetric experiments, ±0.23 is the standard deviation, and (3.75%) is the percentage error

behaviour. Because of the very low pH, that is a limiting factor for microbial activity, soil 2 is a complex example.

- Values of Q_t confirm previous statements, since soil 2 presents a very stable behaviour in its potential productivity along the year in all the calorimetric parameters, despite the apparent differences in Q and P_t.

Figure 2 shows P–t plots for the 3 soils corresponding to spring. As it can be seen, these plots show different shape. This fact makes the shape of the P-t curves to become a very useful tool for studies of soil productivity and state of health [8, 11, 12, 24, 25].

Figure 3 shows microbial activity evolution in the 3 soils over the year. Analysis of this figure allows to observe the different behaviour of microbial

activity over the year, as a consequence of the influence of environmental features, mainly temperature and moisture content.

Conclusions

Microbial activity and soil behaviour greatly depend both on environmental conditions (pH, temperature and moisture) and the forest cover. This study provides interesting data for the design of a rational and sustainable exploitation of the wood resources contained in the different forest formation and also of the agriculture resources of the zone.

Microcalorimetry shows as a very reliable method to assess microbial activity in soils. Results obtained by the microcalorimetric method are in close agreement with those obtained through different physical, chemical and biological tests. The advantage of microcalorimetry over the other techniques is based on the continuous monitoring of soil microbial activity, that provides a real and direct information that leads to productivity potential determination, without the use of other complicated methods that provide only indirect information.

The results achieved through the present study show that the procedure here proposed has been successfully checked using different kinds of soils, both in origin and use, situated in different zones of Galicia. In our opinion, this procedure could be used on any soil everywhere, providing the determination of the own parameters of soil and zones.

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