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DESIGN OF AN EXPERIMENTAL PROCEDURE TO ASSESS SOIL HEALTH STATE*

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The design of a rigorous experimental procedure is the basis for any environmental study. In this work, the basic criteria are established for determination of soil health using microcalorimetry as the main technique complemented by the study of physical (temperature, moisture, porosity, hydraulic conductivity, density and plasticity), chemical (pH and C to N ratio) and biological features (most probable number of microorganisms and organic matter content), and also environmental properties in the form of bioclimatic diagrams. The design was elaborated using as a reference a humic eutrophic-Cambisol subjected to afforestation with *P. pinaster* Aiton situated in Viveiro (Galicia, NW Spain). Main results of this study refer to total heat evolved during the processes (2.65 to 3.81 J g^{-1}), time to reach the maximum of the peak from 16.17 to 19.29 h, and microbial growth rate constant from 0.0732 to 0.1043 h^{-1}. These results change over the year as they are influenced by the action of environmental parameters over soil microbial activity. The results are in close agreement with some others previously reported using different experimental techniques.

Keywords: *environmental study, experimental procedure, microcalorimetry, soil health state*

Introduction

Soil can be considered as an open three-phase physical mean, highly complex, dynamic and in continuous evolution that is influenced by environmental, anthropic and endogenous features.

From the last 1960's, FAO (Food and Agricultural Organization) declared soil and its vegetal cover as an item to be protected as a support of the world society welfare. This was the starting point for a scientific development of soil quality at social, political, economical and environmental levels. Soil quality, or soil health, can be defined as the integration of the innate soil chemical, physical and biological attributes within a framework of space, time and land use. Because of this, soil quality can be used as an indicator of soil capacity to develop in a sustainable way its basic properties that go from the mean where the active growth of vegetal takes place, a key for agriculture productivity, to a water purifier system [1], key for the distribution and the improvement of the quality of this very important and limited natural resource.

The design of the experimental procedure here presented is based on a set of specific assumptions [2]:

- Soil quality is the direct responsible for the capacity to generate and maintain agriculture performance.
- Soil productivity directly depends on main features that are common to every kind of soil: physical fertility and own fertility.
- Microorganisms are real bioindicators of soil quality.
- Soil living phase evolution, and thus the study of microbial activity in soils, can be quantified by using microcalorimetry [3–8].
- Microbial activity is strongly influenced by environmental features, mainly humidity and temperature.

From the ecological point of view, the study of a soil begins with a precise and detailed examination of it as a whole and also of the special characteristics of its surroundings, from climate conditions to vegetal cover or geological substractum [9–11]. Because of this, the microcalorimetric study of soil microbial activity must be complemented with a detailed analysis of those features having influence on this activity. Among these features, the following must be quoted:

- Soil physical properties that show the immediate soil response before external disturbances that affect mainly soil structure. These disturbances can influence on redistribution of water, matter, gases and energy inside the soil. Among these properties one points out: temperature, moisture, field capacity, porosity, hydraulic conductivity, texture, structure, density, residual moisture, plasticity index, compactibility and adhesivity.

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- Chemical properties, responsible for internal processes that modify physical fertility and own soil fertility, such as: elemental composition and C to N ratio.
- Biological properties, mainly related to organic matter content and the most number probable of microorganisms (MPN).
- Climatic conditions: temperature, humidity, evapotranspiration residual evapotranspiration, hydric availability or bioclimatic intensities that are recorded in the form of bioclimatic diagrams.

Analysis of microbial activity must be carried out considering also all the features previously mentioned. This joined study requires the design and development of a very rigorous experimental procedure [8, 10, 11] to be used for every soil and conditions. The global study of microbial activity evolution in soils subjected to very changeable climate conditions, as it happens with Galician soils need the samples being collected in the four different seasonal periods.

This study was made on a humic-eutrophic Cambisol covered by forest residues originated from 2 reforestations with *P. sylvestris* L. in the last 20 years. The sampling zone was situated in a valley with an altitude of about 100 m, protected from winds, surrounded by pine and eucalyptus forest, with trees about 25 m high, little sunny with high humidity and oriented to north. Soil surface is poor in vegetative cover, with little forest residues, very stony, and based on slate.

Experimental

Materials and methods

The procedure here designed is a complex one and, because of this, it was divided into different phases: sampling, laboratory preparation of samples, determination of physical, chemical and biological properties and environmental features and calorimetric experiments.

Sampling

First stage in the experimental procedure was to settle standard statistical criteria that allow the choice of a representative sample for every kind of soil. After 10 years, it was found an experimental procedure for the sampling stage that can be summarized [7, 8, 10–13] in the following points:

- Description and environmental characterization of the zone and geological substractum.
- Choice of the plot where the study is to be made with a minimal surface area of 100 m^2. This area is considered to be ideal for Galician soils; however, it could be changed depending on type of soil, zone or on the complexity of the study to be done.
- Division of the sampling zone in 1 m^2 plots and choice of 6 of them according to statistical criteria after elimination of those situated in the border (edge effect).
- After removal of the very top soil layer, where the vegetable cover is situated, samples were collected to a depth of about 15 cm. All samples from one site were mixed and sieved. The sample was reduced through a coning and quartering procedure. By doing so, the sample was highly homogenized, thus allowing to obtain reproducible and representative results [14].
- 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 procedure previously described, two different kinds of samples were taken, one for microcalorimetric measurements (400 g), named sample 1, and the other for determination of physical, chemical and biological parameters (10 kg), named sample 2.

Sample preparation

Once in the laboratory, moisture content was determined as the mass loss after drying of the sample in a natural desiccating oven, at 105°C [15], to constant mass. Samples were taken off the polyethylene bags and extended on special trays where they were left for drying at a stable room temperature of 20°C during 72 h for subsequent determination of residual moisture following the method above described.

Once dried, samples were sieved using a R72 (mesh size 2×2 mm^2) and the different fractions were characterized according to grain size. The most thin fraction was used for texture determination through Boyoucos method. These data together with field determinations, allow characterization of soil structure. The fraction with mesh size greater than 2 mm was discarded, because even it plays a role in soil global behaviour, this soil is not used for any subsequent test.

Samples to be used for calorimetric experiment (named sample 1) of about 200 g were then placed in hermetically closed polyethylene bags and left in the laboratory, at 4°C, for up to 3 months to ensure reproducibility of measurements [16] before being used for the calorimetric experiments. The remaining sample (sample 2) of about 10 kg, was used for determination of physical, chemical and biological features.

Determination of physical, chemical and biological properties and environmental features

All physical, chemical and biological properties were determined through normalized tests. The following determinations were made:

- Soil pH was determined using a pH-meter. The measurements were performed introducing the electrode in the supernatant solution prepared using 10 g of soil and 25 mL of water [17].
- Water-holding capacity was determined by using a glass tube fitted with a fritted glass disc in the bottom being immersed in water [18].
- Texture. This soil property were determined using Boyoucos hydrometer method following ASTM Standard 152H [19]. These values are shown in the texture triangle ISSS/FAO following the standards proposed by the USDA classification scheme [20].
- Actual density was determined by picnometry [21, 22].
- Bulk density was determined, as it was mentioned above, by the cylinder method [20, 22].
- Moisture and residual moisture were determined in the laboratory by the gravimetric method [16, 23].
- Porosity was determined from data above reported [24].
- Plasticity index was calculated after determination of the soil liquid limit, using the Casagrande Apparatus method (425 μm BS ASTM 4318 or BS 1377:2) and the soil plastic limit by the Atterberg method, in which the sample is sieved (sieve no. 40 ASTM, mesh 0.1 mm). The soil was classified using the plasticity chart, ASTM-D2487 [20, 21].
- Determination of the optimum moisture content for compactibility, and the maximum degree of dry density by the Proctor test BS1377 part. 4, 2.5 kg Rammer method [22].
- Adhesivity was determined by the nickel spatula method [25].
- Hydraulic conductivity constant and infiltration rate were determined using the constant head permeameter, very similar to that designed and used by Darcy and O'Neal. It has a pore 0 and diameter 6 cm funnel following the classification proposed by Landon [26].

Climatic characteristics are presented in the form of a bioclimatic diagram [27, 28]. This diagram is very helpful for the understanding of the influence of climatological features on the soil living phase and the soil productive capacity as a function of the steady vegetative activity of the vegetation growing in this particular zone. Interpretation of the potential bioclimatic intensity is very important, since it shows greater values when both temperature and hydric availability reach the ideal values what in Galicia correspond to spring and beginning of summer.

Calorimetric experiments

After being stored for three months at a 4°C [12], calorimetric experiments were performed using a microcalorimeter 2277 Thermal Activity Monitor [29]. Measurements were carried out in hermetically sealed 5 mL stainless steel ampoules. Microcalorimetric measurements were made using a closed ampoule method [13]. Soil samples of 1 g size were treated with 1.25 mg of glucose g^{-1} soil [7] as a carbon source to assure a microcalorimetric activity capable of being measured. Experiments were repeated four times. The reference ampoule was filled with 1 mL of distilled water [30, 31]. Calorimetric results showing soil behavior were reported in the form of power–time curves. All power–time plots show the four phases common to microbial growth: latency phase, exponential growth, steady phase, and dead phase [8, 11]. A detailed analysis of the shape of the different phases provide a very valuable information about soil behaviour. The main features analysed from microcalorimetric experiments are:

- Q (J g^{-1}) is the total heat evolved during the processes. The greater Q, the greater the microbial activity and thus the greater soil productivity.
- Peak time, P_t (h), is the time to reach the maximum of the peak. This feature is related to soil reactivity, in the sense that the less P_t, the greater the soil activity, and thus more dynamic is soil. As a rule, soils where microbial community, climate, and forest cover maintain together for years, that is soils close to 'climax', use to be more reactive. From the agriculture point of view this is very favourable, but in case of contamination the effect can become devastating.
- Q_t (J g^{-1}), is the heat evolved up to the maximum of the power–time curve. This parameter combines the previous 2. High quality soils must present high Q_t in the lowest possible time.
- μ (h^{-1}), is the microbial growth rate constant. It is directly related to P_t, as the greater μ, the greater should be P_t.

Results and discussion

The results obtained for physical, chemical, and biological properties from the different tests are recorded in Table 1. From these results, it follows:

- Values of pH are in the range from 4.2 to 4.9. These values are strongly acid and could generate a possible toxicity from Al^{3+} and the excess of Co, Cu, Fe, Mn and Zn because of their high mobility in these conditions and also the deficiency in Ca, K, N, Mg, Mo, P and S [32, 33]. This fact limits soil productivity and it can be used only by acidophilic species. These pH values are higher than those found for soils covered by stable pine forest not subjected to forest exploitation that are in the range from 3.3 to 4.6. The abuse of lime to improve pH trying to reach values close to 6 originates unfavourable

Table 1 Physical, chemical and biological properties corresponding to the soil studied

	Spring	Summer	Autumn	Winter
pH	4.2	4.9	4.9	4.2
Temperature/°C	12.0	16.0	14.3	11.8
Moisture/%	22.5	13.5	19.3	24.9
MPN (microorganisms/g soil)	$0.22\cdot10^8$	$0.21\cdot10^8$	$0.70\cdot10^8$	$0.17\cdot10^8$
Texture	Loam			
Actual density/kg m^{-3}	1790			
Apparent density/kg m^{-3}	580 sponge organic type			
Residual moisture/%	3.4			
Total porosity/%	67.6 excessive			
Air porosity/%	49.1			
Water porosity/%	18.5			
Plasticity index	little plastic ML/OL (silt organic soil)			
Adherence/kg m^{-2}	0.5 to 1.5 grain			
Humidity percentage to achieve the ideal compaction/%	20.8			
Hydraulic conductivity constant, K/m s^{-1}	$3.9\cdot10^{-3}$ excessive			
C/N	10			
Organic matter	4.4			
Field capacity/%	32.2			
Structure with abundant stones and diameter between 1 and 3 cm	not 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

phenomenon of compaction and also cationic interchange capacity block that make the soil improductive as the consequence of an excess of calcium in the soil solution.

- Soil temperature is rather stable over the year with an average value of 13.5°C. Taking into account that for Galician soils a temperature over 20°C is a limiting temperature for microbial growth, it can be considered that the average temperature is adequate and not limiting for microbial activity. In adjacent soils the average temperature is about 2°C greater because of their stable vegetable cover. The soil used for this study was lacking in vegetable cover because it was subjected to abundant silviculture tasks to ensure viability of wood exploitation.
- Average humidity is lower than usual values as a consequence of the decrease in rain suffered in Galicia in 2003. Humidity values use to be greater than water-holding capacity (32.2%). Even so, humidity has adequate values and except for periods with very high temperatures, this is not a limiting factor for microbial activity.
- After analysis of humidity values, it can be seen that these values are high because of the great organic matter content that favours water retention along soil profile. This causes also adequate values for residual humidity.
- Porosity, 67.6%, shows a high value which is adequate and typical of a free soil. Also the ratio between water and air porosities is the adequate one. The high value of porosity make the hydraulic conductivity, $3.9\cdot10^{-3}$ m s^{-1}, to be excessive. Even so, compared to adjacent zones not subjected to anthropic activities, this hydraulic conductivity is low. Zones covered by pine, eucalypthus or oak forests present hydraulic conductivities of $4.10\cdot10^{-3}$, $8.9\cdot10^{-3}$ and $5.3\cdot10^{-3}$ m s^{-1}, respectively.
- The different silviculture works have originated a loss of surface structure but, even so, soil can be considered as useful for agriculture performance. Soil texture is loam-type that defines the little plastic nature of this soil that is classified as a ML-OL, silt organic soil.

- As regards to density, the value, 580 kg m^{-3}, of the bulk density defines this soil as a sponge organic type and because of this useful for agriculture exploitation. The actual density presents values that correspond to the slate substractum prevailing in this kind of soil.
- The value of humidity necessary to reach maximum compaction is around 20.8%. This value is rather adequate after analysis of the remaining properties and, in particular texture.
- Low value of adhesivity classify this soil as grain. This is an expression of the joined action of the remaining properties and shows the predominance of soil structuring forces, cohesive forces, over the adhesive forces that originate soil loss of structure [34].
- The ratio C to N is 10. This value shows an equilibrium between humification and mineralization for this soil [35]. Total nitrogen was determined by Kjeldahl method and elementary composition by a Carlo Erba analyzer [36]. This kind of soils need constant attention to avoid irreversible mineralization and because of this, soil productivity needs the constant addition of organic matter and some other kinds of amendments. This soil could not support by itself the constant growth of a wooded area and could originate a scrubland.
- Values of MPN were determined by the presence or absence of microorganisms in several individual aliquots of each of several consecutive dilutions of soil or other material [37]. Values of MPN are in the range from $0.17\cdot10^8$ microorganisms g^{-1} soil, in winter, to $0.7\cdot10^8$ microorganisms g^{-1} soil in summer. These values are very poor and correspond to inappropriate health soils, also, these values are very influenced by soil pH, that is one of the main limiting factors for microbial activity, together with temperature and humidity.
- The organic matter content can be consider as an excessive one and because of this, it is not a limiting factor for soil quality or microbial activity in it. A high content in organic matter, mean value greater than 4%, is a general characteristic of Galician soils.

From the results corresponding to physical, chemical and biological tests and their joined analysis with bioclimatic diagrams, it could be said that microbial activity in these soils is not specially high and also that environmental features have a small influence in these activities as a consequence of the stable climate of the zone and also of the peculiarities of the sampling zone.

To be sure of these statements, a microcalorimetric study was carried out. Table 2 shows results of the main features analyzed in these calorimetric experiments:

- Total heat, Q, is relatively lower than values shown by different soils in this zone. It can be seen that a typical Atlantic forest situated in this zone, that was used as reference, shows a mean value Q=3.06 J g^{-1}, without addition of any kind of amendments while Q value for the soil here studied was 3.25 J g^{-1}. Compared to the heat evolved from soils covered by pines, 2.69 J g^{-1}, the value here presented is much higher as pH is a limiting factor that is very acid for soils covered by pine species.
- The addition of amendments make soil behaviour very reactive thus originating very low P_t values. P_t for this soil, 18 h, is very low compared to values 21 and 28 h presented by the Atlantic forest or the stable pine forest respectively.
- The previous point is in agreement with μ values that are around 0.090 h^{-1}.

The joined study of physical, chemical, biological and environmental features together with microcalorimeter results allows to conclude that the quality of this soil need the addition of amendments in the form of nutrients or pH stabilizers.

Figure 1 is a power–time plot of the soil corresponding to spring. In it can be seen:

- There is a small latency phase very peculiar and marked.
- Exponential growth phase has a great slope that makes the curve to be very sharp.

Table 2 Characteristic microcalorimetric parameters

	Spring	Summer	Autumn	Winter
Q/J g^{-1}	3.81±0.14 (3.66%)	3.62±0.09 (2.37%)	2.93±0.05 (1.75%)	2.65±0.05 (1.97%)
P_t/h	16.17±0.51 (3.18%)	16.17±0.51 (3.18%)	19.29±0.44 (2.57%)	18.37±0.44 (2.37%)
Q_t/J g^{-1}	2.73±0.04 (1.61%)	2.67±0.12 (4.35%)	2.26±0.08 (3.66%)	1.95±0.07 (3.39%)
μ/h^{-1}	0.1043±0.0041 (3.88%)	0.0935±0.0038 (4.09%)	0.0910±0.0026 (2.85%)	0.0732±0.0022 (3.00%)

Q (J g^{-1}) – total heat evolved during the processes; peak time, P_t (h) – time to reach the maximum of the peak; Q_t (J g^{-1}) – total heat evolved up to the maximum of the power–time curve; μ (h^{-1}) – microbial growth rate constant. The final result is 3.81±0.14 (3.66%), where 3.81 is the mean value calculated from 4 microcalorimetric experiments, ±0.14 is the standard deviation, and (3.66%) is the percentage error

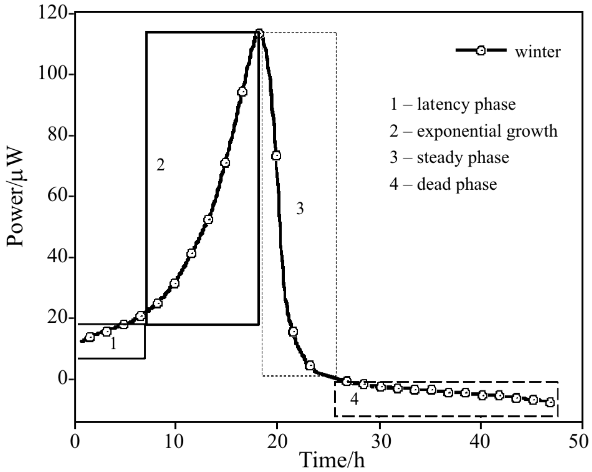

Fig. 1 Power–time curve corresponding to winter. This plot shows the four different phases of microbial growth [8, 11]: latency phase, exponential growth, steady phase, and dead phase

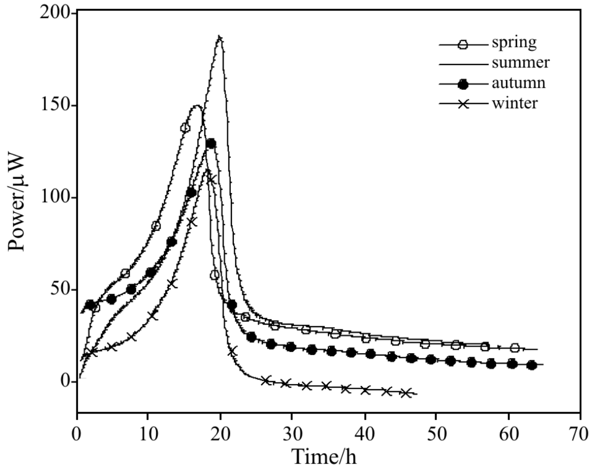

Fig. 2 Power–time curve showing the evolution over the year of microbial activity

- Exponential and death phases are very symmetric and fast.
- The steady phase is a very short one.

Figure 2 allows the analysis of microbial activity in soil over the year. This plot also shows that the maximum microbial activity corresponds to summer in which temperature presents adequate values that are lower in winter.

Conclusions

The study of the evolution of microbial activity using the microcalorimetric technique provides very adequate information about soils quality. The results obtained through this technique are in reasonable good agreement with those obtained using other more complicated equipments [38].

The combined study of physical, chemical, biological and environmental features provides rapid and suitable information about microbial activities in soils.

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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