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The effect of soil moisture on soil microbial activity studied by microcalorimetry

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Abstract

Microcalorimetry has been used to study the effect of moisture on soil microbial activity. Different moisture regimes produce changes on heat flow rate–time curves recorded for soil samples, affecting also certain parameters, such as the total heat evolution Q_{tot}, the microbial growth rate constant μ and growth yield Y, which are calculated by the microcalorimetric method. A positive correlation was found between the percentage humidity, the total heat evolution and the microbial growth rate constant. The values of these parameters decrease with the dryness of the soil. Field capacity moisture appears to be the optimum moisture level for soil microbial activity.

Keywords: Microbe; Microcalorimetry; Moisture; Soil

1. Introduction

The importance of soil moisture in regulating microbial activity in soil is well known [1,2]. Moisture influences a number of physico-chemical properties of soil, such as redox potential, pH, and O_2 and CO_2 levels [3], which in turn influence the microbial population and its activity.

Most studies concerning soil moisture and microbial population relationships are conducted on monthly and seasonal sampling regimes using as indices of soil microbial activity the CO_2 evolution and the dehydrogenase activity [4].

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Microcalorimetry is one of the methods that can be applied to study microbial activity in soils. This method has the advantages that it is specific only to the initial and final energy states of a system and is independent of organisms and of reaction pathway. The heat output is derived largely from the catabolic breakdown of substrate, anabolic reactions contributing little to the overall balance [5]. Microcalorimetry is therefore a suitable technique for assessing the overall catabolism of diverse microorganisms in a complex medium such as soil.

In this paper we study the effect of moisture on the population and activity of soil microbes (over short-term intervals)by the microcalorimetric method in order to establish the relationships between heat evolution and the microbial activity of soil samples with different moisture regimes.

2. Materials and methods

Soil samples were collected from a forest in "El Pedroso" (Santiago de Compostela, Spain). Some of their properties are listed in Table 1. Sampling was carried out at about 10 randomly chosen points from each site. 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 (mesh size 2 × 2 mm), and water content, organic matter and pH were measured.

For the experiments, soil samples were brought to different humidity percentages in the laboratory:

Water content of soil after sampling 15%. Field capacity humidity of soil 29%. Humidity of dry soil 5%. Soil was air dried at room temperature (25°C) for five days. Humidity of waterlogged soil 80%. The soil was over-saturated, leaving a 0.5 cm layer of water on the surface.

The calorimetric system was a Model 2277 Thermal Activity Monitor (Thermometric, AB, Sweden), which is a commercial version of the system developed by Suurkuusk and Wadsö [6]. Experiments were performed in hermetically closed 5 ml stainless steel ampoules. Baselines were recorded from 1 g of soil without nutrient solution in order to quantify the heat evolution of soil samples with different water contents. Heat flow rate–time curves were recorded for 1 g soil samples with different humidity percentages. These samples were amended with 1.25 mg of glucose in a volume of 0.1 ml of distilled water to study the effect of humidity on microbial growth.

The number of living bacteria in soil was counted by the most probable number method.

Table 1
Soil characteristics

Water-holding capacity 24.33%	C/N 15%
Content of carbon 10.27%	pH 4
Content of nitrogen 0.69%	

3. Results

Heat flow rate–time curves recorded from soil samples amended with glucose are shown in Fig. 1. The heat flow of soils with humidity levels of 5%, 15% and 29% increased exponentially after a lag phase and, finally, the microcalorimetric signal output decreased when the glucose had been totally exhausted. The heat flow of the waterlogged soil was much lower than that of the other soils.

In order to express these results in a more quantitative way, the total heat evolution of samples without glucose Q_{tot} and the total heat evolution of samples amended with glucose $Q_{tot,glc}$ were calculated from the areas delimited by the heat flow rate–time curves. Estimates were also made of the values for peak time (time in which the microcalorimetric signal output reaches the maximum amplitude), the microbial growth rate constant μ calculated from the semilogarithmic conversion of heat flow rate [7], the average heat evolution per unit cell formed q, and the growth yield Y. The values of Q_{tot}, $Q_{tot,glc}$ and peak time are shown in Table 2. The total heat evolution of samples without glucose Q_{tot} varied with humidity. Fig. 2 shows a positive correlation between these two variables. In contrast, the total heat evolution of samples amended with glucose $Q_{tot,glc}$ did not show changes related to humidity. Values of this parameter were much higher and less variable than values of Q_{tot}, possibly because the quantity of glucose added was the same for all soil samples.

The value of the peak time also changed with humidity but no positive correlation was found. The maximum value of peak time corresponded to soil samples with 15% humidity, and the lowest value was calculated for soil samples that had been air dried. The value of peak time is related to the number of microorganisms

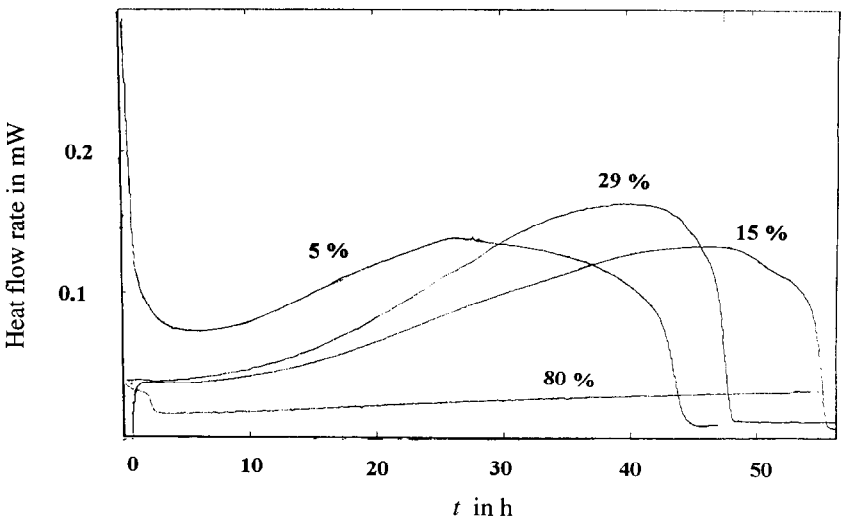

Fig. 1. Heat flow rate–time curves recorded for soil samples amended with glucose, with different humidity percentages. The water content of the soil produces changes in the slopes of the curves.

Table 2
Values of total heat evolution Q_{tot}, total heat evolution of soil samples amended with glucose $Q_{tot,glc}$, and peak time calculated for soil samples with different water contents

Humidity/%	Q_{tot}/(J g^{-1})	$Q_{tot,glc}$/(J g^{-1})	Peak time/h
80	−0.051	1.236 ± 0.339	ND
29	3.131 ± 0.013	12.741 ± 0.644	37.80 ± 1.34
15	1.347 ± 0.040	13.586 ± 0.297	44.30 ± 1.17
5	0.734 ± 0.012	12.172 ± 0.654	28.20 ± 0.69

Mean ± SD, $n = 5$. ND = not determined.

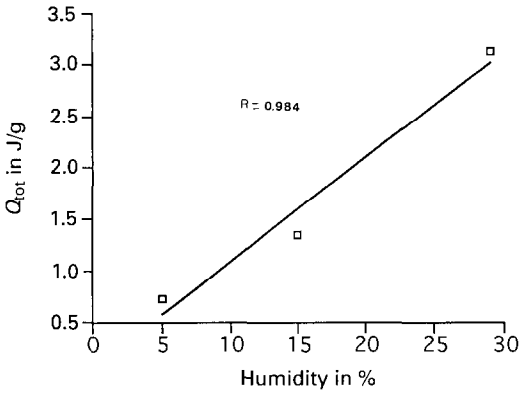

Fig. 2. The total heat evolution Q_{tot} calculated from soil samples with different water contents is plotted against humidity percentage. A positive correlation is found between these parameters except for the waterlogged soil.

in a culture [7], increasing as microbial density decreases, which suggests that the microbial density of the samples with 15% humidity is lower than microbial density at field capacity. The value of peak time was not calculated for waterlogged soil because this sample did not show an exponential increase of heat flow after the addition of glucose.

Table 3 shows the number of microorganisms in the soil samples calculated by the most probable number method. It also shows the values of microbial growth rate constant μ calculated from the slope of the straight lines obtained from the semilogarithmic conversion of heat flow rate and the values for generation time g. Again, it was not possible to calculate these parameters for waterlogged soils. Samples with humidity of 15% showed a lower number of microorganisms than samples at field capacity (29%), which agrees with the values of peak time calculated for these samples, as explained above. The lowest microbial density corresponded to waterlogged soil. The number of microorganisms in these samples was only 5.5×10^4 microorganisms per g of soil. For microcalorimetric measure-

Table 3
Number of microorganisms, microbial growth rate constant μ and generation time g calculated for soil samples with different water contents

Humidity/%	Number of microorganisms per g	μ/h^{-1}	g/h
80	5.5×10^4	ND	ND
29	8.6×10^8	0.048 ± 0.001	14.48 ± 0.36
15	1.4×10^8	0.040 ± 0.001	17.27 ± 0.34
5	ND	0.031 ± 0.002	22.27 ± 1.01

Mean $\pm$ SD, $n = 5$.

ments, it is necessary that the microbial density of cultures be $\approx 10^6$ microorganisms per g. The low microbial density of these samples could be the reason for the low heat flow rate recorded for the waterlogged soils.

It was not possible to quantify the number of microorganisms in samples with a humidity of 5%. In these samples, the addition of glucose produced a rapid increase of microbial population in the cultures, which prevented counting.

The value of the microbial growth rate constant μ was affected by humidity, and here also a positive correlation was found between these two variables (Fig. 3).

Table 4 shows the values of α, q and Y calculated for soil samples with different water contents. The value α of the heat evolution per mol of glucose degraded was calculated from the equation [8]

$$\alpha = \frac{Q_{\text{tot,glc}}}{S_0} \tag{1}$$

where $Q_{\text{tot,glc}}$ is the total heat evolution of soil samples amended with glucose and S_0 is the initial concentration of glucose. The lowest value of α was calculated for soil samples with 5% humidity. This fact suggests than in these samples a higher proportion of energy was diverted for the production of biomass, which is in agreement with the rapid increase of the microbial population found in these cultures after the addition of glucose.

The average heat evolution per unit cell formed q was calculated for samples at field capacity and 15% humidity from the slope of the straight lines obtained by

Table 4
Values of the average heat evolution per mol of glucose degraded α, average heat evolution per unit cell formed q, and growth yield Y

Humidity/%	α/(kJ per mol glc)	q/J per cell	Y/(cells per mol glc)
80	ND	ND	ND
29	1437.59 ± 57.22	$(0.051 \pm 0.001) \times 10^{-8}$	$(2.79 \pm 0.16) \times 10^{15}$
15	1685.74 ± 75.09	$(0.380 \pm 0.020) \times 10^{-8}$	$(4.41 \pm 0.28) \times 10^{14}$
5	1053.99 ± 21.78	ND	ND

Mean $\pm$ SD, $n = 5$.

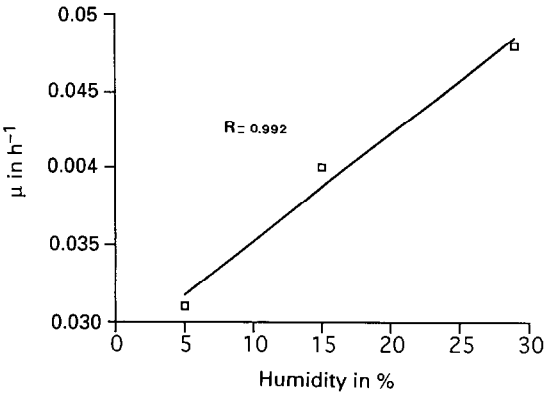

Fig. 3. Relationship between microbial growth rate constant calculated for soil samples amended with glucose and percentage humidity. A positive correlation is found between these parameters.

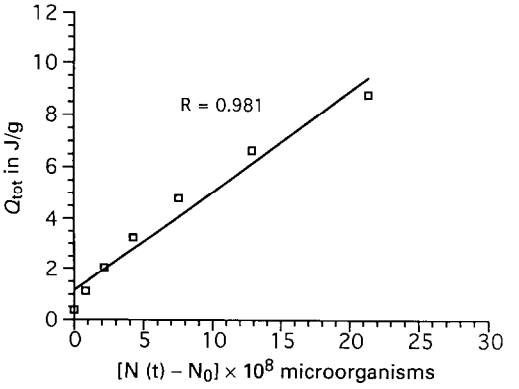

Fig. 4. Correlation between the increase in the number of microorganisms and the heat evolution Q of soil samples with 15% humidity.

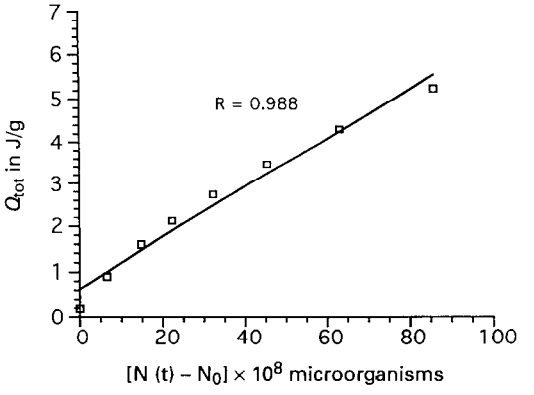

Fig. 5. Correlation between the increase in the number of microorganisms and the heat evolution Q of soil samples at field capacity humidity.

plotting the heat evolution Q against the increase in the number of microorganisms $N(t) - N_0$ (Figs. 4 and 5). It was not possible to calculate these parameters for waterlogged or dry soil because the percentages of humidity in these instances affected the correlations that are usually found between heat evolution and microbial growth [8].

From the equation [8]

$$Y = \frac{\alpha}{q} \tag{2}$$

the growth yield Y was calculated. The growth yield was higher in soils at field capacity.

4. Discussion

The microbial activity of soils varied with humidity. The heat evolution of soil samples without nutrient solution increased with increased moisture, suggesting that dryness inhibits the microbial activity because the heat production of microbes in soil is a direct measurement of the cellular metabolic activity [9–11]. This relationship found between heat evolution and humidity agrees with the relationships between moisture and CO_2 evolution [12,13], dehydrogenase activity [4,14] and ATP production [14,15] reported by different authors.

The addition of glucose induced in most samples (except the waterlogged soil) a rapid and exponential increase of heat flow rate, probably because the addition of this nutrient activates a higher proportion of soil biomass than in normal conditions. The microbial growth rate constant μ and the number of microorganisms also decreased with decreased moisture content [16,17].

The addition of glucose to air-dried soils (5% humidity) induced a high rate of microbial growth in the cultures, but these samples showed the lowest microbial growth rate constant μ calculated from heat flow rate–time curves. These findings suggest that dryness is unfavourable towards most bacteria and few could survive in the air-dried soil, and that these few respond to a favourable change of their environment by rapid reactivation. These factors are reflected on the heat flow rate–time curve as a rapid increase of heat flow rate and a low value of peak time [18].

The waterlogged soil is the sample that shows the lowest value of Q_{tot} and $Q_{tot,glc}$ and the lowest microbial population. The addition of glucose induced only a small increase in the heat flow rate. Anaerobiosis developed in the waterlogged soils, which inhibits aerobic respiration, probably reduces the heat evolution [19].

Field capacity moisture appears to be the optimum moisture level. Samples with this moisture level showed the most favourable values of total heat evolution Q_{tot}, microbial growth rate constant μ and growth yield Y. It is suggested that, in comparative studies on soils using the heat evolution as an index of activity, the soils should be brought to the same moisture level, preferably to field capacity, before starting the experiments in order to obtain comparable results. In this paper

we have also demonstrated that microcalorimetry can be an useful method to study the way in which the microbial activity of soils is affected by environmental factors.

5. Terminology

g	generation time, h
N_0	number of microorganisms before adding glucose
$N(t)$	number of microorganisms at time t
$N(t) - N_0$	increase in number of microorganisms in soils amended with glucose
Peak time	time in which the microcalorimetric signal output reaches its maximum amplitude, h
q	average heat evolution in joules per cell formed
Q	heat evolution joules per gram of soil sample
Q_{tot}	total heat evolution in joules per gram of soil sample
$Q_{tot,glc}$	total heat evolution in joules per gram of soil sample amended with glucose
Y	growth yield in cells formed per mole of glucose degraded
α	average heat evolution per mole of glucose degraded, kJ/(mol glc)
μ	microbial growth rate constant, h

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