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Sensitivity of calorimetric indicators of soil microbial activity

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Abstract

Calorimetry was applied to develop a comparative study between the basal respiration and the degradation of an external carbon source in several soil samples. The main goals were to search for the connection between these reactions in soils and to test the sensitivity of calorimetric indices for the microbial activity. The soil samples were from the Brazilian Amazon and from Galicia (Northwest Spain). The reactions monitored were the aerobic degradation of the organic matter and the aerobic degradation of glucose. The results showed that both reactions are closely correlated when they were studied by specific indices related to the biomass, and that the calorimetric indices calculated were sensitive to different intrinsic soil properties.

Keywords: Calorimetry; Calorimetric indexes; Microbial activity; Soil

1. Introduction

The study of soil microbial activity is very well-known because it has to face great difficulties. The complexity arises from interactions between a large number of biological and physical factors. The understanding of these interactions is very important for the prediction of the microbial quality that must be focused on the soil biomass and its metabolic activity. Many investigations have been developed in that sense [1–3], but on the one hand, the connection between biomass and activity is not well defined yet [4,5] and, on the other hand, these studies are still strongly limited by the methodology [6].

It is necessary to add organic substrates to the soil and to measure the subsequent microbiological response to study the link between biomass and activity. Glucose is the most widely used substrate and it constitutes the basis of most methods to assess the potential activity of microbial communities. But it is widely recognised that the extent to which substrate-induced responses can be related to actual soil processes is limited, because soil microorganisms in situ are rarely confronted with single compounds, and single substrate additions may overlook the complexity of interactions between substrates [7]. It has not been studied in depth yet, whether the study of the glucose degradation reaction in soil can inform about the intrinsic organic matter degradation, and if there is some connection between the kinetics of these two processes. We wonder if calorimetry can provide some information about that topic. It would be very useful to study this fact for two reasons. Firstly, many of the biological methods are based on the response of the soil biomass to the addition of glucose. Secondly, the calorimetric models to measure the efficiency of the soil metabolic reactions quantitatively can be applied only if glucose is added as substrate. The idea in this paper is to provide some information about these items by the use of calorimetric indices. They have started to be applied in soil research [8–10] but curiously, publications are missing about their sensitivity to detect microbial and metabolic activity changes. This is important since any new indicator must be sensitive to the activity and biomass changes related to the soil properties. Calorimetry has the advantage also to study the microbial activity from a thermodynamic point of view. The lack of information about the thermodynamics of the soil microbial reactions makes the method very attractive by itself. It even provides some other advantages, such as a much simpler method than the biological ones [11,12] and gives faster results. Recent evidence demonstrates that thermodynamics can provide also the connection between biomass and activity in terms of thermal efficiency, in a qualitative way and even quantitatively by the application of mass and energy balances [13]. But this method uses glucose too to yield that information.

In this paper, calorimetric data obtained from soil samples with and without glucose were analyzed and compared in order

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to see if the glucose and the organic matter degradation reactions are connected in some way. An attempt was made also to settle which factors could affect the kinetics of both reactions in order to check the sensitivity of the calorimetric indices employed to the different intrinsic and environmental soil properties.

2. Experimental

2.1. Soil samples

Samples were collected in Galicia and in the Amazon. Samples from Galicia (Northwest Spain) correspond to a deciduous forest, *Gpf*, to a pine forest, *Gp* and to a vineyard arable land, *Gv*. In the Amazon, two sites were chosen for sampling in Nova Airao, 200 km from Manaus (Amazonian state of Brazil), on the Rio Negro. The first site *P* is close to the riverside. Samples from location *P* correspond to a primary forest, *Ppf*, to an igarape (plot of land seasonally flooded by the river), *Pi*, and to a Cassava plantation, *Pc*. The second site is named location *T* and is situated inland. Samples from location *T* correspond to a primary forest, *Tpf*, to an orange-lemon grove, *To-l*, and to a pasture, *Tp*. All the details related to the sampling, manipulation of the samples for measurements, and soil properties can be found in previous papers [8,12].

2.2. Microcalorimetric measurements

The applied calorimeter was a TAM 2277 (Thermometric AB, Sweden). This instrument has a four-channel system in which the sample and the reference are introduced simultaneously in a thermostatic cylinder. All the calorimetric measurements were performed at 25 °C. Soil samples were always previously kept at that temperature for 24 h before measurements in a thermostatic room. The basal respiration was recorded as power–time curves from 1 g of sample introduced to a 5 ml stainless steel ampoule. Distilled water 1 ml of was used as reference. The recordings were performed under these conditions for 48 h as was reported in a previous paper [12].

The microbial activity stimulated by the addition of glucose was also measured as power–time curves. For that purpose, 1 g of soil was amended with 0.2 ml of a nutrient solution containing 1.5 mg of glucose and 1.5 mg of ammonium sulphate and introduced to a 5 ml stainless steel ampoule. One gram of soil amended with 0.2 ml of distilled water was used as reference. This procedure has been shown also in previous papers [8,14].

2.3. Calculation of the mass specific heat rate, $J_{Q/S}$

The mass specific heat rate of soil, $J_{Q/S}$, is defined as the heat released by the basal respiration of the soil microorganisms. It is expressed in Joules per gram of soil and day, J g^{-1} d^{-1}. It can be obtained directly by the integration of the power–time curves registered from 1 g of un-amended soil to show the whole activity per unit of time [12].

2.4. Calculation of the total heat evolution, Q_T, of the samples amended with glucose

The total heat evolution, Q_T, was calculated from the area limited by the power–time curves recorded for the samples amended with glucose. It is shown in Joules per gram of soil, J g^{-1} [8,14].

2.5. Calculation of the cell specific metabolic heat rate, $J_{Q/N}$

The cell specific metabolic heat rate, $J_{Q/N}$, is the quotient of the soil mass specific heat rate, $J_{Q/S}$, and the number of microorganisms per gram of soil, N_0. It is given in Joules per cell and day, J $cell^{-1}$ d^{-1}. It shows the quantity of heat that is dissipated per unit of cell and day by the basal microbial respiration [12].

2.6. Calculation of the heat yield, $Y_{Q/X}$

The heat yield, $Y_{Q/X}$, is defined as the quotient between the total heat released by a metabolic process, Q_T, and the increment in the number of microorganisms, ΔN, associated with that process. It is well-known that the addition of single carbon sources to a microbial population causes an exponential microbial growth concomitant to the degradation of the carbon source. The whole process releases heat and it can be quantified as the heat yield which is considered as an empirical measure of the efficiency of the microbial growth reaction [15]. It can be calculated for soils from the power–time curves [14,16].

2.7. Estimation of the soil biomass

The initial number of microorganisms of the soil samples was calculated by the most probable number method, isolating microorganisms in soil extract medium [17].

2.8. Analysis of the results

Three replicates of each experimental measurement were done to show the results as the average and standard deviation. The quantitative calorimetric indexes were compared by one-way ANOVA. Associations among the indexes and soil properties were studied by single and multiple graphical regression analysis.

3. Results and discussion

Fig. 1 represents the values of the mass specific heat rate, $J_{Q/S}$, and the total heat evolution, Q_T, of the samples amended with glucose and ammonium sulphate. It can be seen that the microbial activity measured as heat is highly variable and that all samples react with an enhanced total heat released after amendment with glucose with the exception of sample *Pc*. The ANOVA shows both indexes (i.e. heat production with and without glucose) are significantly different at the 0.05 level. Soils from Galicia have lower values of $J_{Q/S}$ than those from the Amazon, but the highest values of Q_T correspond to samples from Galicia

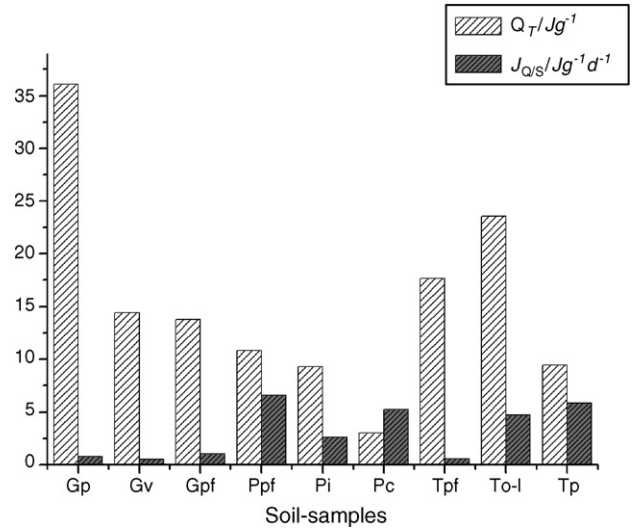

Fig. 1. Plot of the values of the total heat released by the soil samples amended with glucose, Q_T, and the values of the heat evolution rate, $J_{Q/S}$, of the same un-amended soils. Samples *Gp*, *Gv* and *Gpf*, correspond to samples collected in Galicia in a pine forest, in a vine yard and in a primary forest, respectively. Samples *Ppf*, *Pi* and *Pc*, were collected in the Amazon. They came from a primary forest, an igarape and a Cassava plantation, respectively, all of them close to the riverside. Samples *Tpf*, *To-l* and *Tp* were from a primary forest, an orange-lemon grove and a pasture in the Amazonian inlands.

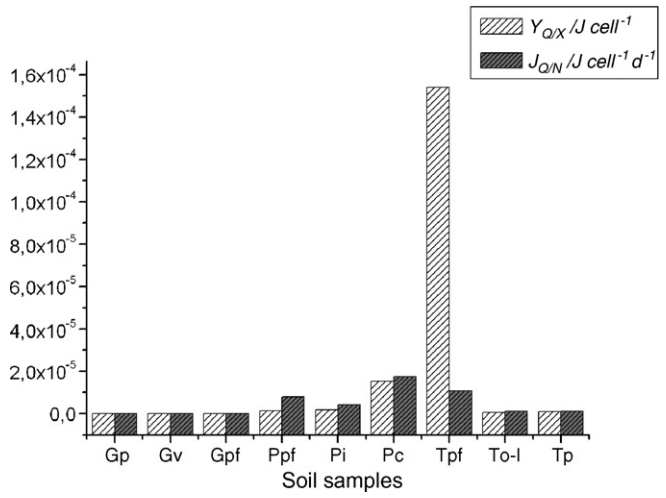

Fig. 2. Plot of the values of the heat yield, $Y_{Q/X}$, calculated from samples amended with glucose, and the values of the cell specific metabolic heat rate, $J_{Q/N}$, calculated for the un-amended samples. The samples are as in Fig. 1.

and those collected in the *T* area in the Amazon. No correlation was found between $J_{Q/S}$ and Q_T.

Table 1 shows some physico-chemical and biological properties of the soil samples. Several of these data have been already published in previous papers [8,12].

Microbial biomass is an important factor in studies of soil microbial activity. It is given in Table 1 as the number of microorganisms per gram of soil, N_0. The microbial population density was lower in the soils from the Amazon than in those from Galicia. No correlation was found between the soil biomass, N_0, and the microbial activity of the samples measured as $J_{Q/S}$ and Q_T. On the whole, samples from Galicia present higher values of biomass and total heat dissipated after addition of glucose than soils from the Amazon, while Amazonian soils appear to have higher values of basal respiration than soils from Galicia.

Fig. 2 shows the values of $J_{Q/N}$ and $Y_{Q/X}$ calculated for all samples. Curiously, differences between $J_{Q/N}$ and $Y_{Q/X}$ data are not as marked as for $J_{Q/S}$ and Q_T, with the exception of sample *Tpf* that presents a high increase in the value of the heat yield, $Y_{Q/X}$, when compared to the other samples. It might be attributed to the low humidity of that sample. The ANOVA indicates here no significant differences between these indexes at the 0.05 level. The biophysical significance of these specific indicators is established in literature [12,18], higher dissipation of heat per unit of cell is linked to a less efficient metabolism.

Figs. 3–5 show the logarithmic correlations found among the values of $Y_{Q/X}$, $J_{Q/N}$ and N_0. The specific microbial activity of the un-amended samples and that from the samples enriched with glucose correlate negatively with the initial number of microorganisms. It seems that increased soil biomass yields a less dissipative metabolism.

The heat yield, $Y_{Q/X}$, is positively correlated to the cell specific metabolic heat rate, $J_{Q/N}$, calculated for the un-amended samples as can be seen in Fig. 5. Both $Y_{Q/X}$ and $J_{Q/N}$ appear to be very sensitive to the soil biomass. The strong connection found with the initial number of microorganims permits an early diagnosis of the soil state from the microbiological data. The main problem now is that none of them give quantitative information about the efficiency of organic matter and glucose degradation.

Table 1
Some biological and physico-chemical properties of the soil samples

Samples	*H* (%)	SOM	C (%)	N (%)	C/N	pH	N_0	Cell-C_{org}
Gpf	27	15.50	9.00	0.45	20.00	4.36	1.90×10^{10}	2.11×10^{11}
Gp	28	10.95	6.35	0.21	30.24	3.78	4.87×10^{9}	7.67×10^{10}
Gv	23	5.47	3.17	0.24	13.21	5.34	7.05×10^{9}	2.22×10^{11}
Ppf	22	6.11	3.90	0.52	7.50	3.41	8.3×10^{5}	2.13×10^{7}
Pi	35	12.90	10.89	0.45	24.20	3.9	6.3×10^{5}	5.78×10^{6}
Pc	29	5.70	4.21	0.74	5.69	3.74	3×10^{5}	7.13×10^{6}
Tpf	6	1.10	1.43	0.05	28.60	3.7	0.52×10^{5}	3.64×10^{6}
To-l	12	2.40	1.55	0.83	1.87	3.75	4.22×10^{5}	2.73×10^{8}
Tp	14	3.30	1.56	0.79	1.98	4.52	5.35×10^{6}	3.43×10^{8}

H, percentage humidity of the soils; SOM, percentage organic matter; C, percentage carbon; N, percentage nitrogen; C/N, carbon to nitrogen ratio; N_0, number of microorganisms per gram of soil wet weight; Cell-C_{org}, number of microorganisms per unit of soil organic carbon.

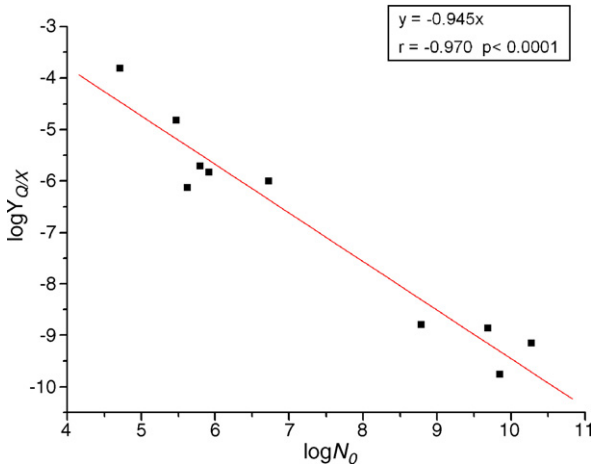

Fig. 3. Logarithmic correlation found between the $Y_{Q/X}$ values of the samples and the initial microbial population, N_0.

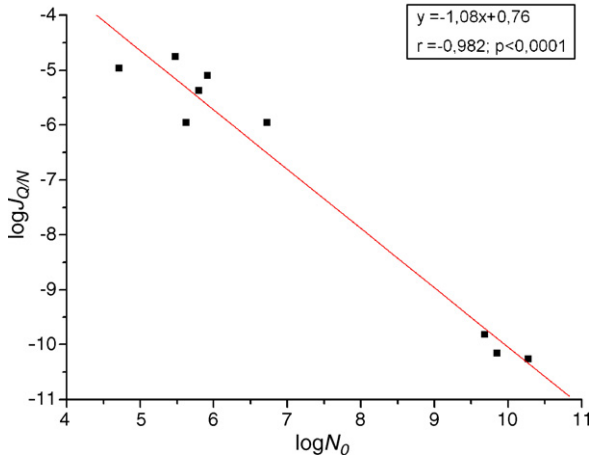

Fig. 4. Logarithmic correlation found between the $J_{Q/N}$ values calculated for the un-amended samples and the initial microbial population, N_0.

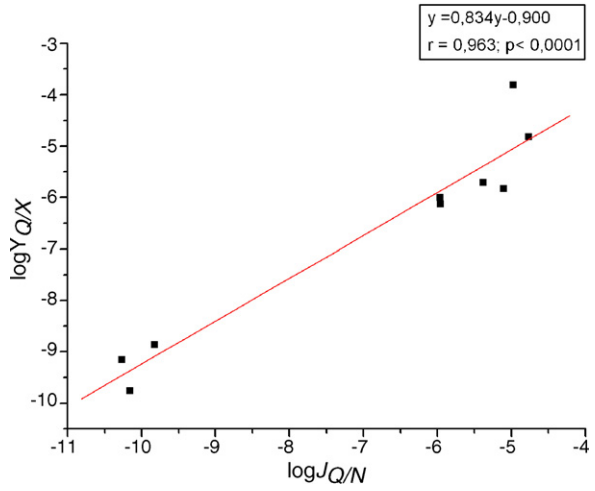

Fig. 5. Logarithmic correlation found between the $Y_{Q/X}$ and the $J_{Q/N}$ data.

That is, it is not possible to know how many carbon units are lost from the soil through respiration and how many remain in the soil. This information is of utmost importance in soil research. A model for the mass and energy balance was shown in a previous paper [13] that gives this information from the $Y_{Q/X}$ data but it cannot be applied to data obtained from un-amended samples. The SOM degradation takes place at zero microbial growth rate (maintenance metabolism), and the activity obtained as heat or CO_2 dissipated is stable. For that reason, it is very difficult to find mathematic relationships to obtain quantitative parameters since the activity indexes (CO_2 or heat) cannot be related to the increment of biomass. Hence, the basal respiration of the soil is studied by the metabolic quotient [19], by the respirometric quotient [20] or by the cell specific heat rate, $J_{Q/N}$ [12]. All these approaches can give only qualitative information about degradation of organic matter. In this paper, the connection found between the $J_{Q/N}$ and $Y_{Q/X}$ values would allow the extrapolation of the quantitative data for the efficiency of the glucose respiration to the organic matter degradation. From the results of the graphical analysis shown in Figs. 3–5 together with the ANOVA evaluation applied to the data shown in Fig. 2, it can be strongly suggested that samples that degrade glucose in a less efficient way, show the same dissipative metabolism when degrading the organic matter. Then, the quantitative data obtained by the mass and energy balances applied to the glucose degradation reaction could be extrapolated to the basal respiration. We could not find information about the existence of this correlation by other methods. It would be necessary to develop more assays in order to see if that connection represents the general behaviour of the microbial activity. That is, if the soil samples that degrade glucose more efficiently in comparative studies, also more efficiently degrade the organic matter. This investigation gives, at least, strong evidence that this connection may exist in nature.

The more significant correlations found between the variables calculated here are shown in Table 2. The indexes of specific microbial activity also closely correlate to the Cell-C_{org} values. This measure is commonly used by biologists because it gives information about carbon availability. The higher biomass per unit of soil carbon is associated with microorganisms that are endowed with a more economic metabolism [21]. Hence, it is considered as an empirical measure of the microbial efficiency to assimilate carbon. The correlation found between $J_{Q/N}$ or $Y_{Q/X}$ and Cells-C_{org} values reinforces their use as qualitative indicators of the efficiency of organic matter and glucose degradation reactions in soils. It seems that samples with higher biomass

Table 2
Results of the single linear and non-linear regression analysis performed with the data involved in this work

log $J_{Q/N}$ vs. log Cell-C_{org}	$r = -0.977$	$p < 0.0001$
log $Y_{Q/X}$ vs. log Cell-C_{org}	$r = -0.969$	$p < 0.0001$
$J_{Q/S}$ vs. N	$r = 0.823$	$p < 0.05$
$J_{Q/S}$ vs. C/N	$r = -0.812$	$p < 0.01$
N_0 vs. SOM	$r = 0.662$	$p < 0.05$
SOM vs. H	$r = 0.814$	$p < 0.01$
SOM vs. C	$r = 0.949$	$p < 0.0001$
C vs. H	$r = 0.845$	$p < 0.01$

Table 3
Results of the multiple regression analysis applied to values of N_0 as the dependent variable and to values of *H*, SOM and C, as the independent variables

R-square (Cod)	Adj. *R*-square	*F* statistic	Prob > *F*
0.796	0.673	6.498	0.035

Table 4
Results of the multiple regression analysis applied to values of $J_{Q/S}$ as the dependent variable and to values of N and C/N as the independent variables

R-square (Cod)	Adj. *R*-square	*F* statistic	Prob > *F*
0.719	0.625	7.679	0.022

per unit of C assimilating more carbon dissipate less energy per unit of cell. Therefore, $J_{Q/N}$ and $Y_{Q/X}$ could be used as an early warning in comparative studies of soil microbial deterioration and perturbation [8,12].

Table 2 also reveals that some of the intrinsic soil properties affect the calorimetric indexes. It is shown as the single regression analysis. In an attempt to discover the whole effect of the intrinsic properties on the biomass and on the activity, a multiple regression analysis was applied to the microbial biomass and to the $J_{Q/S}$ values. The results can be seen in Tables 3 and 4. They show that the SOM, carbon, C, and humidity percentages of the samples are responsible for 67% of the variance of the soil microbial biomass that is connected to $J_{Q/N}$ and $Y_{Q/X}$. The nitrogen percentage, N, and the carbon to nitrogen ratio, C/N (Table 4) are responsible for 62% of the variance of $J_{Q/S}$. Therefore, the $J_{Q/S}$ values do not depend on the size of the biomass but on the N content and the C/N ratio. This is in agreement with other studies that reflect the importance of the nitrogen availability for microbial metabolism [22]. The results strongly suggest that the specific calorimetric indicators of soil microbial activity give more information about the changes in the soil biomass, while the heat rate recorded from un-amended samples appears to inform about the nitrogen availability of the soil. No correlation was found involving Q_T after glucose amendments. Studies reporting the kinetics of glucose degradation in soils show a very close positive relation between the heat evolution rate during the degradation of glucose and the rate of microbial growth [14,16]. This suggests that the values of Q_T could be associated with the fraction of the microbial biomass that is activated for growth by the presence of glucose. That fact has been used by others for soil active biomass determinations [23]. Therefore, Q_T is important for the quantification of some indexes as explained here, but it seems that it only would represent a fraction of the activated biomass growth.

4. Conclusions

The basal respiration of the soil measured as the $J_{Q/S}$ values is sensitive to some intrinsic properties of the soil and depends on the nitrogen availability and on the carbon to nitrogen ratio, while the soil biomass is directly influenced by the C, SOM and humidity of the samples.

The calorimetric specific indexes of microbial activity, $J_{Q/N}$ and $Y_{Q/X}$, are highly dependent on the initial biomass. Therefore, they are indirectly affected by the C, SOM and humidity properties that rule the biomass.

The efficiency of the glucose degradation reaction measured as $Y_{Q/X}$ is closely correlated to the efficiency of the organic matter degradation, $J_{Q/N}$. Then, the data obtained for glucose amended samples could be extrapolated to the organic matter degradation kinetics.

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