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Microcalorimetric determination of the cell specific heat rate in soils: relationship with the soil microbial population and biophysic significance

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Abstract

Microcalorimetry was applied to study the basal respiration in several soils collected in Galicia (Northwest Spain) and in the Brazilian Amazon. The microbial activity was recorded microcalorimetrically as power–time lines during 24 h. The soil mass specific heat rate $J_{Q/S}$ and the cell specific heat rate $J_{Q/N}$ were calculated, and compared to the microbial population of the soil samples and to the number of microorganisms per organic carbon. Results showed an inverse hyperbolic relation between $J_{Q/N}$ and number of microorganisms of the samples, and between $J_{Q/N}$ and the number of microorganisms per organic carbon. The microcalorimetric indexes of microbial activity were affected by some other soil properties, as percent of carbon, nitrogen, and C/N ratio, as well as by the introduction of agriculture, which affected the microbial population. We believe that the cell specific heat rate can be considered as an index that indicates the efficiency of the energy utilization by soil microorganisms, similarly to the specific respiration activity. The reason of its negative correlation with the microbial density could be attributed to changes in the strategy of the energy utilization by microorganisms in soils.

Keywords: Microcalorimetry; Basal respiration; Cell specific heat rate; Soil microbial population

1. Introduction

Soil microorganisms are responsible for the interchange of energy and matter between the soil and the environment. They play an important role in the regulation of the carbon cycle. For that reason, scientists have been looking for the way to measure the energy expenditure by the basal respiration in soils, with the aim of understanding the processes involving degradation of organic matter. The importance of these processes for soil research has led scientists to look for methodologies that allow us to measure the microbial soil activity.

The existence of a highly heterogeneous population in soils implies that any methodology to measure its activity must be largely non-specific in order to include the contribution from all the diverse groups of soil microorganisms. It must be able to detect activity and to quantify it in some way. This is not easy because of the complexity of the soil system. For this reason, the studies about the soil microbial activity have been always limited by the methodologies and techniques employed [1,2].

CO_2 is one of the products of the metabolism of soil microorganisms and it is considered as one of the most important indexes of activity, since it is a direct

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measure of the degradation of the soil organic matter and an indirect measure of the energy expenditure. Nevertheless, it would be interesting to have a direct measure of the energy involved in the basal respiration of soils, since any new method reporting new indexes and higher sensitivity would welcome in this area of research.

Microcalorimetry has good potential to assess the overall biomass and activity of soils [1,2]. It has the big advantage of providing direct measurements of the energy expenditure in terms of heat. As CO_2, heat is a direct product of the degradation of the soil organic matter. It has been reported that heat correlates positively with the CO_2 evolution [1,3]. Hence, heat can be also considered as an index of soil microbial activity. Currently, microcalorimeters are sensitive enough to detect very low values of heat and its application in studies of soil microbial activity has shown that it can provide important quantitative indexes of activity like the microbial growth rate constant, the heat yield of the microbial growth and the enthalpy of the degradation of a carbon source [4–6]. Some other advantages of this methodology in contrast with the study of the CO_2 evolution are

- The high sensitivity of the method permits us to use very small quantities of soil for the determinations (1 g in contrast to 50–100 g used by the CO_2 evolution method).
- Microcalorimeters permit to record continuously the heat evolution of the soil samples as power–time lines.
- Preparation of the samples is very easy.

In this work, we have used microcalorimetry to study the basal respiration of several soils collected in Galicia (Northwest of Spain) and in Amazonian State of Brazil. Our aim is to provide, from the heat evolution rate, an index that can inform about the efficiency of the soil organic matter degradation. Basal respiration in soils is defined as the overall activity or energy spent by the indigenous microbial pool when they degrade the soil organic matter [7]. Since organic matter degradation is a property of all heterotrophs, the basal respiration is commonly used to indicate the level of microbial activity and it is usually measured from the CO_2 evolution or O_2 uptake. The rate at which CO_2 is released varies greatly with soil type and it is affected strongly by the environmental conditions. The breakdown of the soil organic matter is accompanied by the synthesis of new microbial cells which brings about an increase in soil of proteins, polysaccharides, and nucleic acids typical of bacterial protoplasm. But growth rate in nature takes an average value of zero over any long period of time, for this reason the activity recorded by different methods, due to the soil organic matter degradation, is very low and stable [8]. This feature is responsible for the lack of indexes that can inform about the efficiency of the soil organic matter degradation in soils. Here efficiency is considered as the effectiveness in converting substrate carbon into cell carbon and it is important to have a diagnosis of the soil microbial quality and loss of soil sustainability [9]. It is not possible to quantify it in quantitative terms because we cannot measure the microbial growth rate and the increment in biomass due to the soil organic matter degradation, since it represents one of the slowest reactions we can find in nature. Anyway, it is important to search bioindicators of soil microbiological quality that can inform about that efficiency in some way. The first approach was the introduction of the quantity of soil biomass per unit of soil organic carbon (SOC; C_{mic} to C_{org}) [10], which can be measured also as the number of cells per unit of SOC depending on the method employed to estimate the soil biomass. It is stated that higher values of C_{mic} to C_{org} mean accumulation of carbon and viceversa. Accumulation of carbon could be reflecting a more efficient metabolism [8]. As it is established that the soil microflora endowed with a more efficient metabolism releases less CO_2 per unit of cell, it could be possible to obtain information about the efficiency of the soil organic matter degradation if we refer the basal respiration rates (CO_2 per unit of soil mass and day) to the corresponding biomass size. The metabolic quotient $q(CO_2)$, is so obtained and it shows the CO_2–C produced per unit of biomass and time. Comparison of microbial communities in quantitative terms would then be possible [11].

We think it is possible to obtain the same information in terms of heat, using microcalorimetry. It would be important since microcalorimetry would permit to develop that kind of studies and to provide an alternative information to those processes that can contribute to a better understanding of the kinetics of the soil organic matter degradation and the environmental conditions affecting those processes.

We can measure the soil microbial activity microcalorimetrically as power–time lines. The integration of those lines referred to 1 g of soil sample yields the soil mass specific heat rate $J_{Q/S}$, in Joules per gram of soil and day. It represents the decay of the soil organic matter and a measure of the basal respiration in soil. If we refer $J_{Q/S}$ to the biomass per gram of soil, we can obtain the cell specific heat rate $J_{Q/N}$, in Joules per cell and day. We can assume the initial number of cells of the soil as a constant since no increment can occurs in short periods of time. Higher dissipation of heat per unit of cell takes place in less efficient processes. Therefore we can compare the results obtained from the different soil samples and to relate the values of $J_{Q/N}$ to different environmental conditions. If it is sensitive to change in the environmental conditions, we could consider $J_{Q/N}$ as a new bioindicator of soil microbiological quality [12].

As the functional relationship between microbial biomass and specific microbial respiration activity is not yet fully understood, we have compared the cell specific heat rate with the number of microorganisms of the soil samples and with the number of microorganisms per organic carbon, cells-C_{org}. We have also studied the dependence of the microbial biomass and heat released on some soil properties such as percent of carbon, percent of nitrogen, humidity, pH, and C/N ratio and how the introduction of agriculture affects these microcalorimetric indexes.

2. Experimental

2.1. Soil samples

Soil samples were collected in Galicia (Northwest of Spain) and in the Amazon (Amazonian State of Brazil). Galicia has a mild and humid Atlantic climate, while the sampling site in the Amazon supports a tropical climate with two seasons, dry, and rainy. There are big differences in terms of average annual temperature between both locations. The Brazilian Amazon mean annual temperature is 31.4 °C while the mean temperature in Galicia is 15 °C.

All the sampling sites of each location, in Galicia and in the Amazon, were close to each other in order to avoid big differences in the soil properties and in climatic patterns. In each location, three sampling sites were chosen. Properties of the soil samples and samples sites can be observed in Table 1. Samples collected in Galicia correspond to decidious forest from the quaternary period to a nearby Pinus forest introduced during the reforestation program in the sixties and to a cultivated field (vineyard). In the Amazon, two sites were chosen for sampling in the same location. That place is called Nova Airao and it is located 200 km far away from Manaus, up Negro River (2°N, 61°W). The first site is close to the river side and it was named as location P. It is affected by the sedimentation of the river and for that reason, it is rich in organic matter content. In location P, three nearby sites were chosen for sampling corresponding to the Amazonian Primary forest and to a 1 year old Cassava plantation introduced after burning the Primary forest.

Table 1
Sampling places and soil samples used in this work[a]

Sampling site	Soil samples	Vegetation
Galicia	Gdf	Decidious forest
	Gp	Pinus forest
	Gv	Vineyard
Brazilian amazon		
Location P	Ppf	Primary forest
	Pi	Igarapé
	Pc	Cassava plantation
Location T	Tpf	Primary forest
	Tol	Orange–lemon trees
	Tp	Pasture

[a] The two locations chosen in the Amazon for sampling, named location P and T, differ in their soil organic matter content. Primary forests and arable lands were chosen in order to see how agriculture affects the soil microbial activity if compared with the Primary forests.

The second site in Nova Airao was named as location T. It is situated inland, some kilometers far away from the river side. In contrast to samples from location P, this new ones were poor in organic matter content. In this location, three nearby sites were chosen for sampling too, corresponding to the Amazonian Primary forests, arable lands, and pasture. In total, nine samples were studied.

All samples were collected from 0 to 10 cm of depth with a sampling corer. Each sample consist of 10 evenly distributed sub-samples mixed together in polyethylene bags. Samples collected in Galicia were brought to the laboratory within 1 day. Samples collected in the Amazon were brought to the laboratory

Table 2
Physic-chemistry properties of the soil samples used in this study[a]

Sample	Humidity (%)	SOM	C (%)	N (%)	C/N	pH
Gdf	27	15.5	9	0.45	20	4.36
Gp	28	10.95	6.35	0.21	30.24	3.78
Gv	23	5.47	3.17	0.24	13.21	5.34
Ppf	22	6.11	3.9	0.52	7.5	3.41
Pi	35	12.9	10.89	0.45	24.2	3.9
Pc	29	5.7	4.21	0.74	5.69	3.74
Tpf	6	1.1	1.43	0.05	28.6	3.7
To-l	12	2.4	1.55	0.83	1.87	3.75
Tp	14	3.3	1.56	0.79	1.98	4.52

[a] SOM represents the percentage of soil organic matter, C and N are the percentages of carbon and nitrogen content while C/N represents the carbon to nitrogen ratio.

within 1 week. During that time, soil was kept in a cooler. In the laboratory, all samples were sieved (2 mm) to remove stones, roots, and plant debris. Subsamples were taken to measure pH and soil organic matter, carbon, nitrogen, and humidity percentages. pH was measured with a Crison micropH-meter 2000 in a solution of 10 g of soil and 25 ml of distilled water. The humidity percentages was measured by soil weight loss after drying during 24 h at 110 °C. The determinations of carbon, nitrogen, and organic matter was performed by oxidative methods using reactants as Mohr salt, sulphur acid, phosphoric acid, and chromium potassium. Results are shown in Table 2. The number of microorganisms of the samples was counted by the most probable number method [13]. The remaining soil was stored at 4 °C in polyethylene bags during 1 month before microcalorimetric measurements.

2.2. Microcalorimetric measurements

The microcalorimeter used was a LKB 2277 thermal activity monitor from thermometric, which is a heat conduction calorimeter.

Soil samples were incubated at 25 °C during 24 h before microcalorimetric measurements. After this period, 1 g wet weight of soil was introduced in a 5 ml calorimeter stainless steel ampoule. One milliliter of distilled water was used as reference. Both ampoules, sample, and reference were introduced simultaneously in the microcalorimetric channel. The basal respiration was registered during 24 h at 25 °C.

2.2.1. Analytical procedures

The heat flow rate ϕ, released due to basal respiration in soils is recorded in microwatts per gram of soil and day and represented as power–time lines. The integration of those lines permits to obtain the total heat released by 1 g of soil sample per day, which can be called soil mass specific heat rate, $J_{Q/S}$ in Joules per gram of soil and day.

The total heat dissipated by the samples can be referred to the biomass size of the soils as the quotient between the total heat per gram of soil and day and the number of microorganisms per gram of soil. We obtain the total heat released per unit of cell and day that can be called cell specific heat rate $J_{Q/N}$, in Joules per cell and day. These data are obtained as the average of three replicas.

Other data reported in this paper is the amount of microorganisms per unit of soil carbon calculated as the quotient between the number of microorganisms and the quantity of SOC. It is given as number of microorganisms per gram of SOC, cells-C_{org}.

All the regression analyses reported in this work were performed using the mean of three replicas.

3. Results

Physic-chemical properties of the soil samples are shown in Table 2. It can be observed that in samples from Galicia (Gdf, Gp, and Gv) reforestation with Pinus and introduction of agriculture (samples Gp and Gv, respectively) appears to decrease the nitrogen content of the samples, while in the Amazon an opposite pattern in values of N is observed. Soils supporting arable lands in the Amazon (Pc and To-l) show higher values of N content than those from Primary forests (Ppf and Tpf) affecting the values of the C/N ratio.

Results from calorimetric measurements together with the microbial density of the samples and values of cells-C_{org} can be observed in Table 3.

3.1. Comparison of the soil mass specific heat rate data, $J_{Q/S}$

Values of $J_{Q/S}$ can be observed in Table 3. The soil mass specific heat rate of the samples collected in Galicia is lower in soils reforested with Pinus and

Table 3
Values of the soil mass specific heat rate $J_{Q/S}$, cell specific heat rate $J_{Q/N}$, number of microorganisms per gram of SOC cells-C_{org}, and number of microorganisms per gram soil wet weight N_0, for the samples used in this study[a]

Samples	$J_{Q/S}$ (J/g day)	$J_{Q/N}$ (J/cell day)	Cells-C_{org}	N_0 (g wet wt.)
Gdf	1.01 ± 0.05	(5.41 ± 0.15) × 10^{-11}	2.11 × 10^{11}	1.9 × 10^{10}
Gp	0.74 ± 0.03	(1.52 ± 0.06) × 10^{-10}	7.67 × 10^{10}	4.87 × 10^{9}
Gv	0.49 ± 0.02	(6.95 ± 0.28) × 10^{-11}	2.22 × 10^{11}	7.05 × 10^{9}
Ppf	6.59 ± 1.02	(7.94 ± 1.23) × 10^{-6}	2.13 × 10^{7}	8.3 × 10^{5}
Pi	2.63 ± 1.53	(4.17 ± 2.4) × 10^{-6}	5.78 × 10^{6}	6.3 × 10^{5}
Pc	5.23 ± 0.88	(1.74 ± 0.29) × 10^{-5}	7.13 × 10^{6}	3 × 10^{5}
Tpf	0.55 ± 0.54	(1.06 ± 0.5) × 10^{-5}	3.64 × 10^{6}	0.52 × 10^{5}
To-l	4.69 ± 0.79	(1.11 ± 0.18) × 10^{-6}	2.73 × 10^{8}	4.22 × 10^{6}
Tp	5.85 ± 1.32	(1.09 ± 0.31) × 10^{-6}	3.43 × 10^{8}	5.35 × 10^{6}

[a] Values are given as Mean ± S.D. ($n = 3$).

in the arable land than in soils from the decidious forest. In location P (Amazon), the seasonal sedimentation in the igarape and the introduction of the Cassava plantation appear to decrease also the soil mass specific heat rate when compared to data obtained from the Primary forest. Samples collected in the igarape, show the lowest value of $J_{Q/S}$. Location T appears to follow a different pattern. Samples collected in the arable land and in the pasture show higher values of $J_{Q/S}$ than samples collected in the Primary forest.

3.2. Comparison of cells-C_{org} and microbial density

In Galicia, samples collected in the Pinus forest (Gp) and in the arable land (Gv) show a lower number of microorganisms than that from the Primary forest (Gdf), while the percentage of microorganisms per organic carbon is much lower in the Pinus forest (see Table 3). Location P in the Amazon shows a similar pattern. Samples affected by sedimentation and agriculture (Pi and Pc) have a lower number of microorganisms than samples collected in the Primary forest (Ppf). The depletion of the microbial density was bigger in the Cassava plantation. The same pattern is observed with the values of cells-C_{org}.

Once again, values from location T differ from the above data. Arable lands (To-l) and pasture (Tp) show the higher values of microbial density and cells-C_{org} compared with those obtained from the Primary forest (Tpf).

3.3. Comparison of the cell specific heat rate data, $J_{Q/N}$

Results of the $J_{Q/N}$ values for all the soil samples are shown in Table 3. Samples collected in the Pinus forest (Gp) show the highest value of $J_{Q/N}$ in Galicia. The arable land (Gv) has a higher value of $J_{Q/N}$ than that from the Primary forest (Gdf) and lower than that from the Pinus forest (Gp). In location P, the sample from the Cassava plantation (Pc) has the highest value of $J_{Q/N}$. The lowest value corresponds to the igarape (Pi). In location T, samples collected in the arable lands and pasture show lower values of $J_{Q/N}$ than that from the Primary forest in that location.

On the whole, it seems that agriculture appears to decrease the soil organic matter content, to deplete the number of microorganisms of the samples and to increase the values of $J_{Q/N}$ calculated by microcalorimetry, with the exception of samples collected in location T in the Amazon which follow a different pattern.

3.4. Correlations

In order to establish the existence of dependence among the variables of this study, linear and non-linear regression analyses were performed with our data. The significant correlation found are shown in Table 4.

Analysis of the dependence between the cell specific heat rate $J_{Q/N}$, and microbial density for all samples, result in an inverse hyperbolic relationship. The results shown in Fig. 1 suggest the following expression:

$$J_{Q/N} = 1.139 N_0^{-1.116} \quad (1)$$

Table 4
Correlation between the indexes of microbial activity reported in this paper and some soil properties[a]

$\log J_{Q/N}$ vs. $\log N_0$	$r = -0.986$	$y = 1.139-1.116x$	S.D. = 0.428	$P<0.001$
$\log J_{Q/N}$ vs. $\log$ cells-C_{org}	$r = -0.977$	$y = 3.174-1.165x$	S.D. = 0.547	$P<0.001$
$J_{Q/S}$ vs. N	$r = 0.823$	$y = -0.482+7.504x$	S.D. = 1.522	$P<0.01$
$J_{Q/S}$ vs. C/N	$r = -0.812$	$y = 5.764-0.181x$	S.D. = 1.563	$P<0.01$
N_0 vs. C	*r = 0.902*	*y = −3.009+2.124x*	*S.D. = 3.57*	*P<0.05*

[a] Data in italics shows the correlation found when soils collected in location P in Amazon were omitted.

The same relationship was obtained plotting $J_{Q/N}$ values vs. cells-C_{org}. Fig. 2 shows the linear fit obtained when the logarithm of the $J_{Q/N}$ values is plotted vs. the logarithm of the cells-C_{org} data, suggesting the equation:

$$J_{Q/N} = 3.174 \times \text{cells-}C_{org}{}^{-1.165} \quad (2)$$

The increase in cells-C_{org} values is interpreted in literature as accumulation of carbon and enhancement of efficiency in soils, which result in a depletion of the $J_{Q/N}$ values as Eq. (2) shows.

Results shown above strongly suggest that the biomass of the soil affects the efficiency of the soil organic matter degradation in terms of cell specific heat rate $J_{Q/N}$.

In a lesser extent, it was found a positive linear correlation between the soil mass specific heat rate $J_{Q/S}$, and the percentage of nitrogen, and a negative correlation between $J_{Q/S}$ and C/N ratio. It seems that nitrogen and C/N ratio play an important role in the basal respiration of the soil.

As it has been established that increase in $q(CO_2)$ is observed in soils with low pH [14], we have plotted our values of $J_{Q/N}$ vs. pH to see if it responds as $q(CO_2)$. The result can be seen in Fig. 3. Now significant correlation was found between the above mentioned data but it can be observed a trend of $J_{Q/N}$ to decrease with enhancement of pH.

If samples collected in location P in the Amazon are not included in the regression analysis, it is obtained

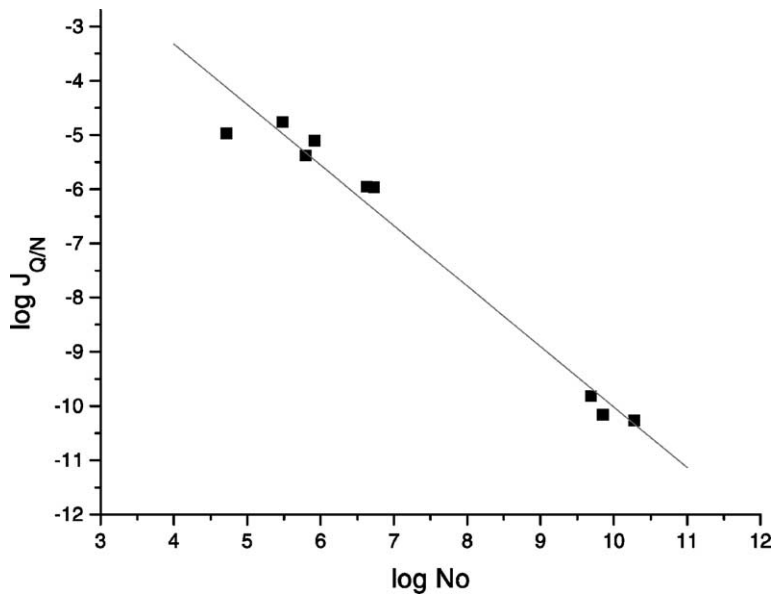

Fig. 1. Plot of the logarithm of the cell specific heat rate $J_{Q/N}$, in Joules per cell and day, against the logarithm of the initial number of microorganisms of the soil samples N_0, in number of cells per gram of soil wet weight.

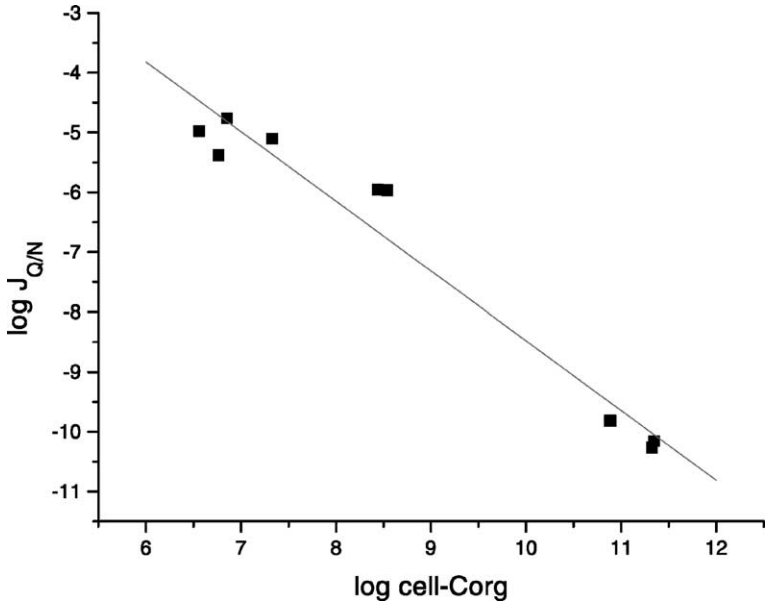

Fig. 2. Plot of the logarithm of the cell specific heat rate $J_{Q/N}$, in Joules per cell and day, against the logarithm of the number of cells per gram of SOC, cells-C_{org}.

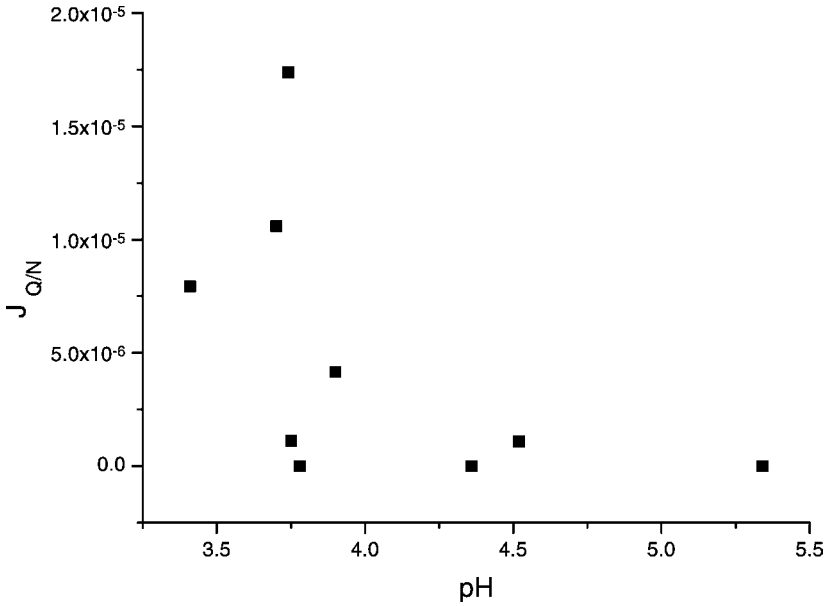

Fig. 3. Plot of the cell specific heat rate values $J_{Q/N}$, against the pH of the soil samples used in this work.

a positive lineal relation between the carbon content of the samples and the number of microorganisms.

No more significant correlations were found.

4. Discussion

Results shows that the basal respiration of soils studied in terms of energy dissipation vary a great deal within samples. The reasons appear really difficult to establish due to the complex nature of soil systems.

Soils from the Amazon showed higher basal respiration in terms of $J_{Q/S}$ and less efficient activity reflected in higher values of the cell specific heat rate $J_{Q/N}$, than soils from Galicia. The reason for that difference could be the higher mean temperature of the Amazonian soils. It has been reported that $q(CO_2)$ increases with temperature [10] and that basal respiration in terms of CO_2 in cooler climates is lower than in warmer climates [11]. Values of $J_{Q/S}$ and $J_{Q/N}$ reported in this paper could follow the same pattern.

A very close negative relation was found between the cell specific heat rate and the number of microorganisms of the samples. Analysis of the dependence between $J_{Q/N}$ and the number of microorganisms resulted in an inverse hyperbolic relation. The relation is consistent with the study of Santruckova and Straskraba [15]. They report a strong negative relation between $q(CO_2)$ (amount of CO_2 released per gram of soil biomass) and microbial biomass. The slope −1.181, that they obtained is very close to that reported in this work −1.116. Although, the relation between specific basal respiration and biomass was also apparent for several studies [14,16] it is not yet well understood. Some authors had written about the existence of spurious correlation [17,18] but in contrast, Prairie and Bird [19] demonstrated that the existence of spurious correlation does not negate the existence of the shape of their regression. Almost all measurements can be considered ratios of some other values; it is common to express values per unit weight, unit time or unit length. Besides, the relation between cell specific heat rate and biomass have been found also in terms of oxygen consumption per cell in aquatic ecosystems [20]. The slope was −0.89, close to our value also. Therefore, the similarity of the relationship between specific microbial activity and population density observed in soil and water using different indexes (O_2, CO_2, and heat) suggests that it has a common origin.

Another index of efficiency of soil microbial activity is the number of microorganisms per organic carbon. Increased values of C_{mic} to C_{org} ratio indicates accumulation of carbon in soil and viceversa [12]. In this work, an inverse hyperbolic relation was found between the cell specific heat rate $J_{Q/N}$, and the number of microorganisms per unit carbon, cells-C_{org}. As Eq. (2) shows, it seems that increased values of cells-C_{org} (that means, accumulation of carbon in soil) diminish the values of $J_{Q/N}$, that is, the cell specific activity in the soil. Therefore, increasing microbial density and cells-C_{org} leads microorganisms to a more economic and efficient metabolism and to dissipate less energy as heat per unit of cell. The same response is observed in studies based on the CO_2 evolution, therefore low microbial biomass values can be characteristic of either stress or disturbance. The Franz Josef Glacier data demonstrates that a high $q(CO_2)$ and a low microbial biomass may both occur reflecting their dual response to underlying stress [11,14]. In this work the cell specific heat rate appears to follow the same pattern. The samples with the lower number of microorganisms, showed the highest values of $J_{Q/N}$, that means, the less efficient metabolism. One of those samples was collected in the Cassava plantation in the Amazon. The roots of Cassava synthesize cyanide, which is poisonous for aerobic metabolism. This fact could cause stress. The other sample was collected in a Primary forest with a low organic matter percentage in the Amazon. As it has been established that soil microbial biomass and biomass C-to-organic C ratios both respond readily to disturbance effects [21,22], their relation to $J_{Q/N}$ values would permit to use this index as an early warning on the deterioration of soil quality and as an alternative measure of changes in microbial biomass in response to disturbance as it was done with $q(CO_2)$. Results show that the introduction of agriculture in the sampling sites of this work, altered the values of $J_{Q/N}$ when compared to those obtained from nearby Primary forests.

The question now is: which factors may affect the microbial density in soil?

Literature reports that microbial biomass depends positively on the SOC content and on soil moisture [14,23]. In this work we find the same relation to SOC, when samples collected in location P are not included

in the correlation analysis. Increasing values of carbon in soil, affect positively the microbial density and diminish the cell specific heat rate. This effect is observed in samples collected in Galicia and in the T area in the Amazon. If we study the samples collected in the P location in the Amazon separately, we observe that the sample with the highest value of SOC (Pi) has a lower number of microorganisms than the sample Ppf with lower SOC value but higher microbial biomass. It is clear that in this area some other environmental factors affect the microbial density. They could be the anaerobiosis developed in the igarapés due to rapid sedimentation during the seasonal flood of Negro River and the existence of cyanide in the soils supporting Cassava. These facts could be affecting the correlation between SOC and microbial biomass.

If we study the soils included in the regression, we observe that in Galicia, reforestation with Pinus and agriculture reduces the microbial biomass, the SOC and increases the cell specific respiration in terms of $J_{Q/N}$ when compared to the Primary forest there. In that sense, Pinus could be responsible of stress. In location T in the Amazon, soils show an opposite pattern. Samples supporting agriculture and pasture show the highest microbial biomass and lowest values of $J_{Q/N}$. The Primary forest in this area had a very low value of SOM and percentage of humidity that could affect the microbial density and activity. The use of fertilizers that increase pH in the arable lands here and the presence of cattle in the pastures, could be responsible of the increase in SOM and SOC observed in these samples, affecting positively the microbial density and for some unknown reason, leading microorganisms to a more efficient metabolism reflected in lower values of $J_{Q/N}$.

In this work, no correlation was found between nitrogen and $J_{Q/N}$ but there is a positive linear relation between the soil mass specific heat rate $J_{Q/S}$, and the nitrogen and a negative linear relation between $J_{Q/S}$ and C/N ratio. This is consistent with other studies that found that microbial activity was correlated to nitrogen at a higher level of significance than with SOC, this stresses the importance of nitrogen availability for microbial metabolism while SOC solely reflects the size of the organic matter pool [24]. No correlation was found between $J_{Q/N}$ and $J_{Q/S}$. The reason could be that both indexes provide different information about microbial activity. Values of $J_{Q/S}$ recorded by microcalorimetry in this work, were not correlated with the microbial biomass in agreement with Santruckova and Straskraba [15] which report that soil respiration was not related to microbial biomass since it remains nearly on the average constant. Nevertheless, basal respiration studied as CO_2 evolution, has been found to be highly correlated with microbial biomass by other authors [25,26] and Sparling [2] reported a positive dependence between heat flow rate in milliwatts and microbial biomass.

In this work, we did not find a consistent relation of the basal respiration and microbial biomass of the samples with the pH. It has been reported that neutral acidification has strong effects on microbial performance in linear forest soil [27] and that increased pH, increases the soil respiration and soil microbial biomass [28]. In our study the effect of pH is not very clear. Fig. 3 shows that $J_{Q/N}$ appears to decrease with increasing pH in an attempt to show if $J_{Q/N}$ responds as $q(CO_2)$, since it has been stabilized that, enhancement of $q(CO_2)$ by low pH values is indicative of stress [27]. The relation reported here is only qualitative. The reason could be that differences in pH among the samples are not strong enough to show their effect.

On the whole, our results suggest that SOC affects the microbial density of the samples, while percent of nitrogen and C/N ratio have a stronger influence on the basal respiration measured as heat. The microbial biomass affected strongly the cell specific heat rate. The increase in the number of microorganisms and the increase of the number of cells per organic carbon reduces the cell specific heat rate. Two possible reasons could be

- If carbon availability is one of the driving variables for the microbial biomass, as it has been reported [14], the organic matter input in soils could have favoured organisms, which are endowed with a more economic metabolism. This fact could explain the enhancement of the microbial population, accompanied by the reduction of $J_{Q/N}$, observed in the samples collected in the arable lands and pastures in location T in the Amazon.
- The increase in the number of microorganisms makes micropopulation of soil to develop a more economic metabolism. As almost any type of biological activity needs energy to meet different environmental conditions and because the available

energy may also be limited, the strategy of energy regulation and the efficiency of energy utilization is of utmost importance for the survival and growth of the different organisms and for the competition or co-operation between these organisms in any natural habitat [29]. In summary, if the microbial population increases in a plot of land for any reason, the cell specific heat rate diminish in an attempt of microorganisms to organize their activity better, since the work they have to do now is shared by a high number of microorganisms.

5. Conclusions

In fact, we believe that the basal respiration and the efficiency of the soil organic matter degradation can be studied in terms of soil mass specific heat rate and in terms of cell specific heat rate, respectively. The application of $J_{Q/N}$, could be performed as an early warning of the deterioration of soil quality and as an alternative measure of changes in microbial biomass. The equations reported here permits to develop models to predict efficiency from the biomass measurements.

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References

[1] G.P. Sparling, Soil Biol. Biochem. 13 (1980) 93.
[2] G.P. Sparling, J. Soil Sci. 34 (1983) 381.
[3] A. Tancho, R. Merckx, R. Schoovaerts, K. Vlassak, Thermochim. Acta 251 (1995) 21.
[4] L. Nuñez, N. Barros, I. Barja, Thermochim. Acta 237 (1994) 73.
[5] N. Barros, S. Feijóo, S. Fernández, J.A. Simoni, C. Airoldi, Thermochim. Acta 356 (2000) 1.
[6] N. Barros, S. Feijóo, A. Simoni, S.A.M. Critter, C. Airoldi, J. Therm. Anal. Cal. 63 (2001) 577.
[7] T.H. Anderson, K.H. Domsch, Soil Biol. Biochem. 22 (1990) 251.
[8] G.H. Wagner, Soil Biochemistry, Marcel Dekker, New York, 1975.
[9] M. Alexander, Introduction to Soil Microbiology, Wiley, New York, 1961.
[10] T.H. Anderson, K.H. Domsch, Zeitschrift für Pflanzenernährung und Bodenkunde 149 (1986) 457.
[11] H. Insam, Soil Biol. Biochem. 4 (1990) 525.
[12] T.H. Anderson, K.H. Domsch, Zeitschrift für Pflanzenernährung und Bodenkunde 149 (1986) 457.
[13] R.Y. Stanier, E.A. Adelberg, J.L. Ingraham, The Microbial World, Prentice-Hall, Englewood Cliffs, NJ, 1985.
[14] D.A. Wardle, A. Ghani, Soil Biol. Biochem. 27 (1995) 1601.
[15] H. Santruckova, M. Straskraba, Soil Biol. Biochem. 23 (1991) 525.
[16] T.H. Anderson, K.H. Domsch, Soil Biol. Biochem. 21 (1989) 471.
[17] S.B. Long, Sociological Methodology: K.F. Schüssler, New York, 1980, 37.
[18] D.E. Weller, Ecol. Monogr. 57 (1987) 23.
[19] Y.Y. Prairie, D.F. Bird, Oecologia 81 (1989) 285.
[20] V. Drabkova, V. Straskrabova, Gidrobiologiecheskie Processy Vodoemach (Ed.), I.M. Raspopov, Leningrad, 1983, 26.
[21] D.S. Powlson, P.C. Brookes, B.T. Christensen, Soil Biol. Biochem. 19 (1987) 159.
[22] D.A. Wardle, G.W. Yeates, R.N. Watson, K.S. Nicholson, Soil Biol. Biochem. 25 (1993) 857.
[23] V. Wolters, R.G. Joergensen, Soil Biol. Biochem. 23 (1991) 897.
[24] E.A. Kaiser, T. Mueller, R.G. Joergensen, R.H. Insam, O. Heinemeyer, Soil Biol. Biochem. 24 (1992) 675.
[25] H. van de Werf, W. Verstraete, Soil Biol. Biochem. 19 (1987) 261.
[26] X. Vekemans, B. Godden, M.J. Pennicks, Soil Biol. Biochem. 21 (1989) 53.
[27] E. Baath, K. Arnebrant, Soil Biol. Biochem. 26 (1994) 995.
[28] D.A. Wardle, Biol. Rev. 67 (1992) 321.
[29] L. Gustaffsson, Thermochim. Acta 251 (1994) 69.